ARTIFICIAL IMPRINTING OF SALMON AND TROUT IN LAKE MICHIGAN

Fish Management Report No. 80
DEPARTMENT OF NATURAL RESOURCES
Madison, Wisconsin
1975

A Cooperative Project of the University of
Wisconsin Sea Grant College Program and the
Wisconsin Department of Natural Resources

ACKNOWLEDGEMENTS

We especially thank and acknowledge the following for their work in the field: Sy Drzeweicki at the South Milwaukee Water Filtration Plant; Bill Boes, Army Corps of Engineers, Kewaunee, Wisconsin; Tim Whitney, Kathy Hughes, Cheryl Gosse, Rod Smith, Terry Chapp, Steve Lewis, Tim Lewis, Peter Johnsen, and James Duffy, field assistants, University of Wisconsin Laboratory of Limnology.

We gratefully acknowledge Andrew Dizon (National Marine Fisheries Service, Honolulu, Hawaii) for advice in all aspects of this study. Aivars Stasko (Fisheries Research Board of Canada, St. Andrews, New Brunswick) assisted in the early phases of its development. Peter Hirsch at the University of Wisconsin Laboratory of Limnology helped to plan some of the experiments.

Numerous Wisconsin Department of Natural Resources personnel were invaluable in supplying logistical support for the marking and transport of young fish and in aiding in the collection of salmon during the spawning season. HATCHERY PERSONNEL: John Klingbiel, Supervisor of Fish propagation; Don Czeskleba, Manager Wild Rose Fish Hatchery; Gerald Kryka, Manager Crystal Springs Fish Hatchery; Robert Housel, Manager Hayward Fish Hatchery; James Meierotto, Manager Brule Fish Hatchery; Dan Hahn, Manager Bayfield Fish Hatchery; Wes Warwick, Manager Nevin Fish Hatchery. We also respectfully thank the crew at Wild Rose Fish Hatchery: Ellwsorth Batten, Everett Eckstein, Dale Grant, Al Mattice, Clair Moore, and Dick Tetzlaff. LAKE MICHIGAN PERSONNEL: Paul Schultz (Plymouth) and James Moore (Sturgeon Bay), Lake Michigan Work Unit Biologists; James Addis, Fish Management Staff Specialist (Milwaukee); James Holzer, Fish Manager (Delafield), James Baumgart (Plymouth) and Terry Hupf (Delafield) deserve special recognition for assistance in the field. LAKE SUPERIOR PERSONNEL: George King, Fish Management Coordinator (Bayfield). Bruce Swanson Fish Manager (Bayfield) has been in charge of the Lake Superior imprinting program. OTHER PERSONNEL: Ken Walker (Spooner); Ted Schwochert (Asylum Bay);Don Dodge (Plymouth).

Ed Mueller and his advanced biology students at South Milwaukee High School helped to fin clip several groups of fish for the South Milwaukee experiments. Graduate students Steve Brandt, Betsy Colburn, Jean Heitz, Sharon Klinger, Jack Robinson, and Sherry Steffel at the University of Wisconsin Laboratory of Limnology also assisted in fin clipping operations.

Al Scidmore and Henry Guckel of the University of Wisconsin Department of Electrical Engineering supplied the ultrasonic transmitters used in the tracking study. Some of the tracking equipment was borrowed from the National Marine Fisheries Service Biological Laboratory, Seattle, Washington. We also acknowledge the United States Coast Guard at Milwaukee, Wisconsin and Two Rivers, Wisconsin, for logistical support.

Doug Weber, Don Park, Wes Ebel, and Jim Mighells, National Marine Fisheries Service, Seattle, Washington, and Roy Wahl, National Marine Fisheries Service, Portland, Oregon, permitted use of their unpublished data.

This project was supported by the University of Wisconsin Sea Grant College Program (National Oceanic and Atmospheric Administration, Dept. of Commerce, Grant NOAA 2-35209), the National Science Foundation (Grants GB7616 and GB343), and the Wisconsin Department of Natural Resources (The Anadromous Fish Act, Grants AFS-8 and AFS-9).

Edited by Betty Les
Graphics by Dennis Leveque

This report is also being issued by the University of Wisconsin Sea Grant College Program as Sea Grant Advisory Report #414, University of Wisconsin, WIS-SG-75-414. All citations of the report should use the Department of Natural Resources Report number.

ARTIFICIAL IMPRINTING OF SALMON AND TROUT IN LAKE MICHIGAN

By

Allan T. Scholz, Ross M. Horrall, Jon C. Cooper,
Arthur D. Hasler and Dale M. Madison
University of Wisconsin, Laboratory of Limnology

and

Ronald J. Poff and Russell I. Daly
Wisconsin Department of Natural Resources

Fish Management Report 80
Wisconsin Department of Natural Resources
Madison, Wisconsin 53701

1975

ABSTRACT

In studies conducted in Lake Michigan from 1971 to 1974, coho salmon and steelhead trout which were exposed to a synthetic chemical (morpholine) while undergoing the smolting process, returned during the spawning migration to a stream which had been scented with morpholine. Behavioral and physiological experiments with homing adult fish showed that morpholine-exposed fish can detect and discriminate morpholine while control fish which had not been exposed did not react to the chemical. A second chemical, phenethyl alcohol, was also used for imprinting and similar results were obtained.

These results document that coho salmon and steelhead trout learn and utilize artificial chemical cues for homestream selection. They indicate that it is possible to manipulate the migration of some species of salmonids by artificially imprinting smolts to synthetic chemicals. Fish do not have to be imprinted to a natural stream system but rather can be imprinted at a fish hatchery and later decoyed or attracted to specific areas. This approach offers greater management flexibility and may also be preferable for economic and biological reasons. These studies have direct application for the management of salmon stocks in Lake Michigan and other parts of the world.

A detailed summary of imprinting techniques is provided which includes information about equipment, selection of chemicals, critical or sensitive exposure periods and stocking procedures. Sample calculations are provided in an appendix.

CONTENTS

INTRODUCTION

Several salmonid fish species native to the west coast of North America have been stocked annually in Lake Michigan since 1966. Principal species stocked are coho salmon (Oncorhynchus kisutch [Walbaum]), chinook salmon (Oncorhynchus tschawytscha [Walbaum]) and steelhead trout (Salmo gairdneri Richardson).

In their native environment, salmonid fishes return with great accuracy to their natal streams to spawn (reviewed by Scheer 1939; Hasler 1966; Harden-Jones 1968). The spawning migration of salmon has been divided into (1) open-water migration when salmon migrate from the open water of oceans or lakes into shore areas near the home river and (2) upstream migration, including location (or recognition) of the main river and home tributary (Hasler 1966; Harden-Jones 1968). There is evidence to indicate that different sensory cues may play important roles in the ocean migration and upstream migration of homing fish (reviewed by Hasler 1966; Harden-Jones 1968; and Hara 1970).

Information about the home stream does not appear to be inherited because young salmon which are taken from their original home stream prior to the downstream migration and transplanted into a second stream will return to the second stream to spawn (Donaldson and Allen 1957; Carlin 1968; Jensen and Duncan 1971; Ricker 1972; Vreeland, et al. 1975). Thus, it seems that homing is connected, at least in part, with a process of rapid and irreversible learning at the time the young salmon begin their seaward migration. This type of behavior, termed "imprinting" (Hasler and Wisby 1951; Brett and Groot 1963; Mayr 1974), has provided the basis for stocking salmon in Lake Michigan.

Lake Michigan streams are not suitable for natural spawning of salmonids because of a lack of suitable gravel substrates, cold winter water temperatures, and large fluctuations in water level (Avery 1974) as well as low dissolved oxygen and poor water quality in some cases. As a result, the salmonid populations of Lake Michigan are maintained predominantly by hatchery released fish. The stocking procedure for coho salmon, for example, has been to hatch and raise the salmon in a hatchery for one and a half years. At this age the fish are transferred to "smolting ponds" located on Lake Michigan tributary streams and held there for about one month (from mid-April to mid-May) in order to "imprint" them to the river system where they are stocked. During the period the fish are held in the ponds they undergo smolt transformation, a process involving physiological and behavioral changes which initiate downstream migration. When the smolting process begins the fish are allowed to migrate from the ponds and down the stream into Lake Michigan. After 18 months in the lake, about 5% of the fish which were originally stocked will return to their respective stream system to spawn with a minimal amount of straying into other streams (Wisconsin Department of Natural Resources unpublished reports).

It has been theorized that during the imprinting period salmon learn the odors of their home stream and subsequently utilize this information to relocate the home stream during the spawning migration (Hasler and Wisby 1951; Hasler 1966). Hasler and Wisby (1951) suggested that it might be possible to imprint salmon artificially by exposing young fish to a synthetic chemical and then later, during the spawning migration, to scent a specific area with this chemical in order to attract or decoy the salmon to a new location.

In 1971, a joint study between the University of Wisconsin Sea Grant Program and the Wisconsin Department of Natural Resources was undertaken to determine if coho salmon could be imprinted to a synthetic chemical. This report deals with work on artificial imprinting of coho salmon conducted between 1971 and 1974 in Lake Michigan. It is organized into three sections. The first section presents the results of artificial imprinting experiments on coho salmon. The second section discusses the management applications of these experiments. The third section is an explanation of the techniques and equipment used to imprint fish. Experiments with other species and sample calculations are described in an appendix.

PART I. IMPRINTING EXPERIMENTS

Investigations on the artificial imprinting of Lake Michigan salmonids involved both the upstream migration and open-water migration phases. The bulk of the work was with coho salmon and will be reported here in detail. Studies conducted with steelhead trout are reported in the appendix.

Three separate methods were used to determine if salmon could be imprinted to a synthetic chemical odor: (1) field census investigations, (2) behavioral (ultrasonic tracking), and (3) physiological (EEG) experiments.

UPSTREAM MIGRATIONS

The basic design for these studies was to expose fingerling coho salmon to a synthetic chemical, morpholine. An equal number of fish were not exposed (controls) and both groups of fish were stocked directly into Lake Michigan. During the spawning migration 18 months later, morpholine was introduced into a river near the stocking location and the number of morpholine-exposed and unexposed fish returning to this simulated home stream was determined. It was hypothesized that if salmon were using odor information to relocate their home stream, then only the fish which were exposed to morpholine would recognize the river as the home stream and return there to spawn. Unexposed fish served as controls for random straying into the stream. Behavioral and physiological experiments were conducted in order to test the specific response of homing fish to the chemical.

Field Census Investigations

Procedures

Coho salmon were hatched and raised at a Wisconsin fish hatchery for about one and a half years. At this age the fish were divided into two equal sized groups, marked with different fin clips and held in separate raceways.

Odor Environment. The same water source (artesian spring water or, alternately, Lake Michigan water) was used to supply both raceways. This water was considered "neutral" because it was not connected with any river draining into Lake Michigan and, therefore, could not provide the homing adult fish with any information about the location of a Lake Michigan tributary stream.

Imprinting chemicals. A synthetic chemical, morpholine ($OCH_2CH_2NHCH_2CH_2$), was metered into one of the raceways (Fig. 1). The fish in the second raceway were not exposed to morpholine and were, therefore, controls for this experiment. Morpholine, an organic compound, was selected for imprinting the salmon because (1) it is not known to occur in natural waters, (2) it is highly soluble in water, (3) it is probably stable in the natural environment and (4) because earlier work (Wisby 1952; Hasler 1966) indicated that this compound could be detected by coho salmon at low concentrations (about 1×10^{-6} mg/l). A steady state concentration of 5×10^{-5} mg/l was selected for imprinting salmon for this experiment so that there could be some degree of fluctuation in water flow (for example, from runoff) without going below a level which could be detected by the fish. A second chemical, phenethyl alcohol ($C_6H_5CH_2OH$), which was used as an imprinting odor for some experiments will be described later.

Imprinting period. The fish were exposed to morpholine for approximately five weeks (from mid-April to mid-May) during their presmolting and smolting stages. Smolting is a development phase in the life cycle of coho salmon when the fish begin to move downstream (Hoar 1951, 1958). This period was chosen because earlier studies (Donaldson and Allen 1957; Jensen and Duncan 1971), demonstrated that fingerling coho salmon which had been taken from their original home stream (or natal hatchery) just prior to smolting and transplanted to a second stream, subsequently returned to the second stream to spawn. Past work had also shown that salmon which were released into Lake Michigan tributary streams during this period returned to spawn in the tributary where they were stocked. Smolting was characterized by loss of parr marks, color changes and an increasing tendency to swim downstream.

Stocking procedures. When most of the fish were observed crowding at the downstream end of the raceway they were transported in a truck to Lake Michigan and released. The

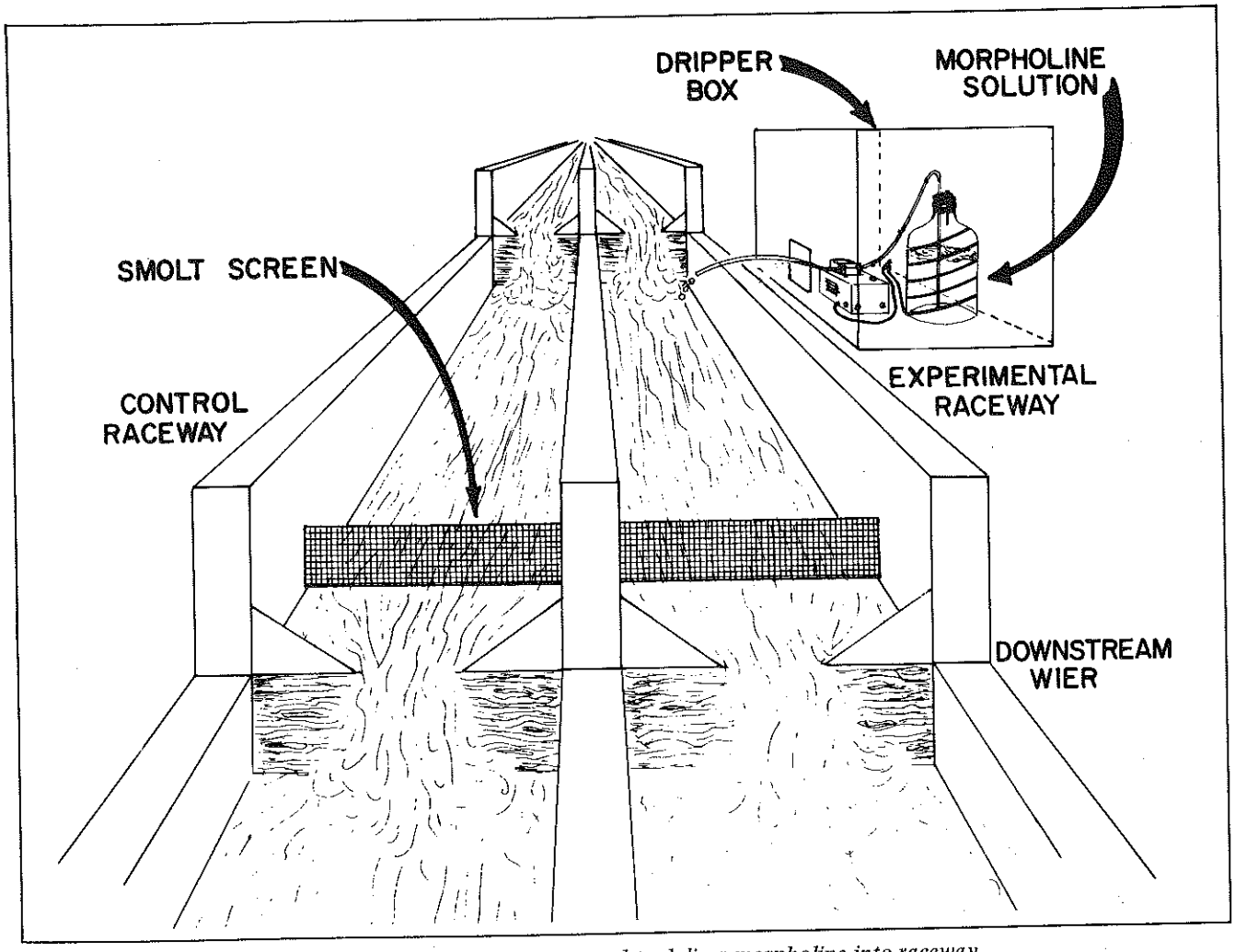


FIGURE 1. Experimental raceways, indicating system used to deliver morpholine into raceway.

stocking locations depended on the experiment and are listed in the results section. In most cases fish were stocked near the mouth of the test stream, that is, the stream which was scented during the spawning migration.

The close proximity of the stocking site to the test stream was considered necessary because it is not known how the fish return to the general area of the home river. The purpose for stocking the fish directly into the lake was to eliminate the downstream migration and thereby reduce the possibility of fish learning information about the test stream.

For most experiments the test stream was Oak Creek in South Milwaukee, Wisconsin (Fig. 2). The census techniques described below were utilized at Oak Creek and those for other locations are described in the results.

Monitoring the return. During the spawning migration 18 months later, morpholine was dripped into Oak Creek at approximately the same concentration to which the fish had been exposed. The concentration was calculated for the mean flow rate to be 5×10^{-5} mg/l. Because of the variable flow rate of the creek and the constant rate of morpholine addition to the stream, the actual stream concentration probably varied between 3×10^{-4} and 1×10^{-5} mg/l. In addition, small amounts of morpholine were added to the stream during periods of increased flow. Adult salmon were collected in Oak Creek by electrofishing and creel census surveys (angler catch). Creel surveys (Fig. 3) were conducted daily along the length of Oak Creek about once every two hours starting at sunrise and continuing until dark, and electroshocking trips were conducted once or twice weekly. Fish were unable to move past a dam situated 1.50 km from the mouth which made census surveys relatively easy because only a small portion of the river had to be monitored. These surveys were continued until no salmon were left in the river and, therefore, we believe that a high proportion of the actual number of salmon which returned to Oak Creek were captured.

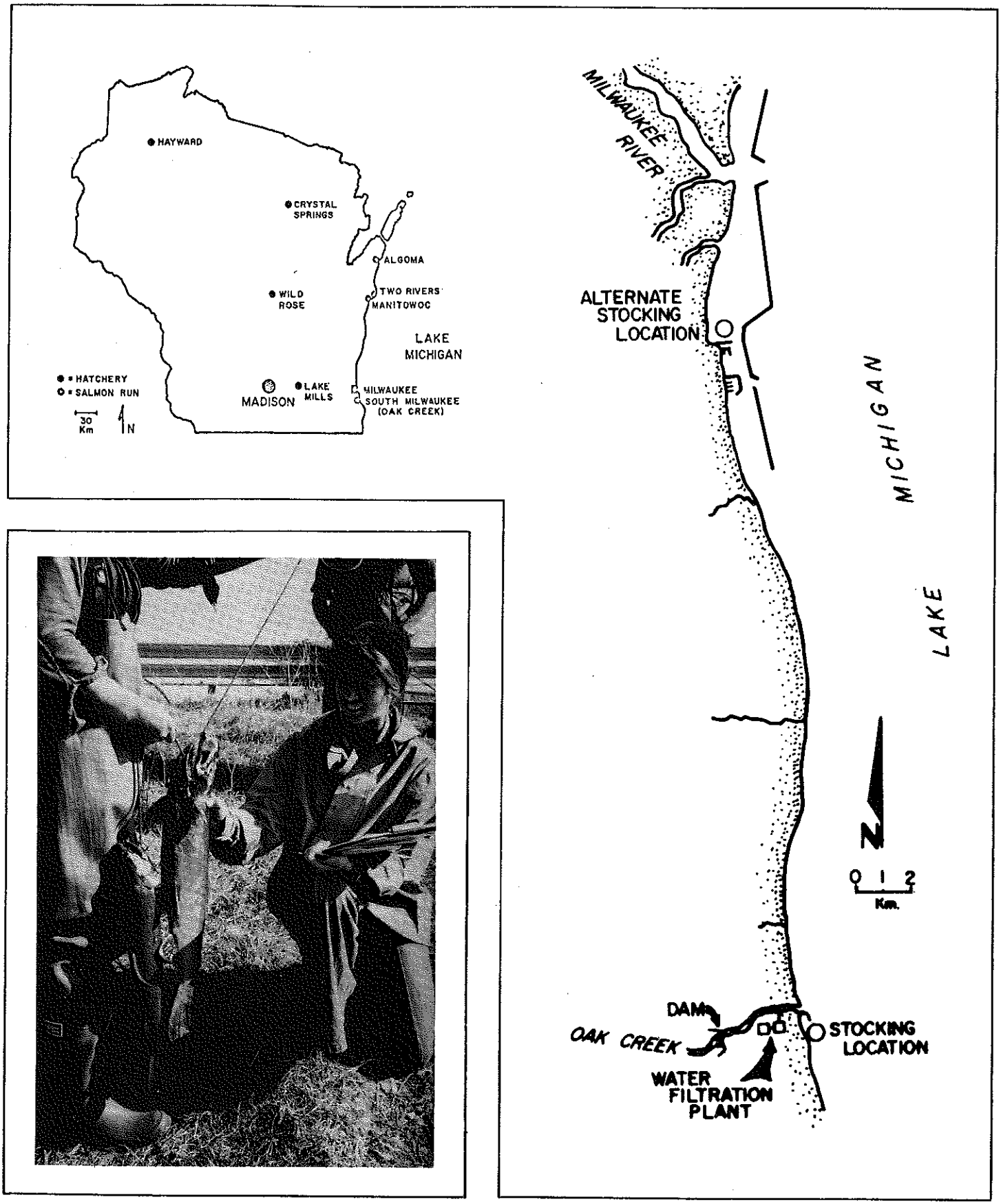


FIGURE 2. Research area in South Milwaukee, Wisconsin, indicating standard and alternate stocking locations. During the spawning season, morpholine was metered into Oak Creek near the water filtration plant. Inset map of Wisconsin shows hatchery locations. Fish were trucked from Wild Rose Fish Hatchery to the stocking site.

FIGURE 3. Examining a fish during a creel census check.

Many situations which could have biased the results of these experiments (i.e. caused differences in the return of imprinted and nonimprinted groups independent of chemical odor) were taken into consideration when planning the experiments. They included:

1. Differences in genetic background and early life history between imprinted and control groups. In these experiments both groups of fish were from similar genetic backgrounds (i.e. eggs from Lake Michigan fish taken from one location) and raised under identical conditions until they were separated.
2. Careless or incorrect marking of young fish. It was assumed that errors made in fin clipping were similar for each group.
3. Differential mortality because of different fin clips. To control for this possibility, paired groups were usually given symmetrical clips. Since the Great Lakes Fishery Commission in Ann Arbor, Michigan, controls the assignment of fin clips in Lake Michigan to avoid duplication of clips, it was not always possible to use symmetrical clips for imprinted and control groups. Fin clips are listed in the results for each experiment. Fish were marked about 40 days before they were released and records were kept of the mortality in each hatchery raceway. Most of the mortality occurred immediately after fin clipping. In all of the experiments described in this report, mortality in the hatchery raceways was low, and it was similar for all groups. Therefore, it was assumed that differential mortality did not bias the results. It is not known if there was a differential mortality caused by marking or other factors after the fish were released into the lake.
4. Regeneration of clipped fins. Rich and Holmes (1929) and Stuart (1958) reported that double fin clips can help reduce problems of identification. For this reason, double fin clips were used in most experiments. It was assumed that regeneration would be similar for each group.

Results - Oak Creek

In April of 1971, 8,000 morpholine-exposed and 8,000 unexposed coho salmon smolts were transported from Wild Rose Fish Hatchery to South Milwaukee, Wisconsin, and released near the mouth of Oak Creek (Fig. 2) (Madison et al. 1973; Scholz et al. 1974; Cooper et al. MS). During the spawning migration in the fall of 1972 morpholine was added to Oak Creek and the stream was monitored for returning fish. A total of 216 morpholine exposed fish (2.68% of the 8,000 fish which were originally stocked) were captured in Oak Creek compared to only 28 controls (Table 1), a ratio of 7.7:1, although a ratio of 1:1 would be expected if morpholine had no effect on the experimental group.

In 1972 this experiment was repeated in order to replicate the 1971 study. Two groups, imprinted and controls, with 5,000 marked fish in each group were released at Oak Creek in the spring of 1972. In the fall of 1973, 437 exposed fish (8.74% of those stocked) and 49 unexposed fish, a ratio of 8.7:1, were recovered at Oak Creek (Table 1) confirming the results of the 1971 experiment.

These recoveries indicated that fish which are exposed to morpholine as smolts will return during the spawning migration to a stream which is scented with morpholine. The number of morpholine treated fish recaptured ranged from 2.7% to 8.7% of the fish originally stocked which compares well with the 5% return of smolting pond fish.

In 1973 a control experiment for the effect of morpholine was conducted. Five thousand morpholine exposed and 5,000 unexposed coho smolts were again released near the mouth of Oak Creek, but this time morpholine was not added to Oak Creek during the fall 1974 spawning season. Fifty-one exposed and 55 unexposed salmon were captured at Oak Creek (Table 2) a ratio of about 1:1. Both exposed and control fish returned in about the same numbers as controls in previous experiments. These results demonstrated that morpholine was an important factor for attracting imprinted fish to Oak Creek.

Results - Manitowoc and Two Rivers

The methods used at Oak Creek were modified and additional experiments were conducted. Three groups of fish were used. One group was exposed to morpholine and a second group to a different chemical, phenethyl alcohol (PEA), at a concentration of 1 X 10-3 mg/l. A third group was left unexposed. All three groups (with 5,000 fish in each group) were stocked in Lake Michigan halfway between the Little Manitowoc River and the Two Rivers Breakwater (Fig. 4). This location was selected, instead of Oak Creek, for stocking

Table 1. Results of artificial imprinting experiments with coho salmon (O. kisutch) conducted at Oak Creek in 1971 and 1972.

Experimental Group	Fin Clip*	Number Released	Date	Number Recovered**	Date	Percent of Fish Stocked
Exposed	D or A+RP	8000	May 71	216	Fall 72	2.68
Controls	LV + RV	8000	May 71	28	Fall 72	0.35
Exposed	RM	5000	May 72	437	Fall 73	8.74
Controls	LM	5000	May 72	49	Fall 73	0.95

* Fin clip abbreviations are: A = adipose; D = dorsal; LV = left ventral; RV = right ventral; LP = left pectoral; RP = right pectoral; LM = left maxillary; RM = right maxillary; a plus sign (+) denotes a double fin clip.
** Includes a small number of jacks recovered during the previous fall.

Table 2. Results of control experiments on artificial imprinting of coho salmon (O. kisutch) conducted at Oak Creek in 1973. Morpholine was not present in Oak Creek during these experiments.

Experimental Group	Fin Clip*	Number Released	Date	Number Recovered	Date	Percent of Fish Stocked
Exposed	A+RP	5,000	May 73	51	Fall 74	1.00
Controls	A+LP	5,000	May 73	55	Fall 74	1.10

* See footnote Table 1 for key to abbreviations.

the fish because PEA could be used for attracting fish to a separate stream from the morpholine fish.

During the spawning migration in the fall of 1974 morpholine was introduced into the Little Manitowoc River and PEA was added at the Two Rivers breakwater. Each of these rivers was about 4.8 km from the original stocking point. Both locations were monitored for returning fish (Fig. 4). In addition to these rivers, 26 other locations were sampled to determine if imprinted fish were straying into nonscented streams and also to determine what happens to control fish (Fig. 4). The amount of effort spent in monitoring each location was different and is indicated in Table 3.

In general, morpholine fish returned to the morpholine stream, PEA fish returned to the PEA stream and control fish were recovered at several locations. These results are summarized in Table 4.

Two hundred and seven morpholine-exposed fish were captured in the morpholine-scented Little Manitowoc River. This represented 4.1% of the fish originally stocked. Thirteen morpholine fish were recovered at other locations. Of the total number of morpholine fish recovered, 94.1% were found in the Little Manitowoc River.

One hundred and thirty-two PEA salmon were captured at Two Rivers, the PEA scented stream. This represents 2.7% of the fish originally stocked. Fourteen PEA fish were captured in other locations. Of the total number of PEA exposed fish censused, 90.5% were recovered at Two Rivers. Of the 133 PEA fish recovered at Two Rivers, 118 were captured where PEA was being metered into the breakwater area (Table 3). Only 15 fish were taken upstream despite considerable effort in sampling, indicating that imprinted fish can be attracted to a very specific area.

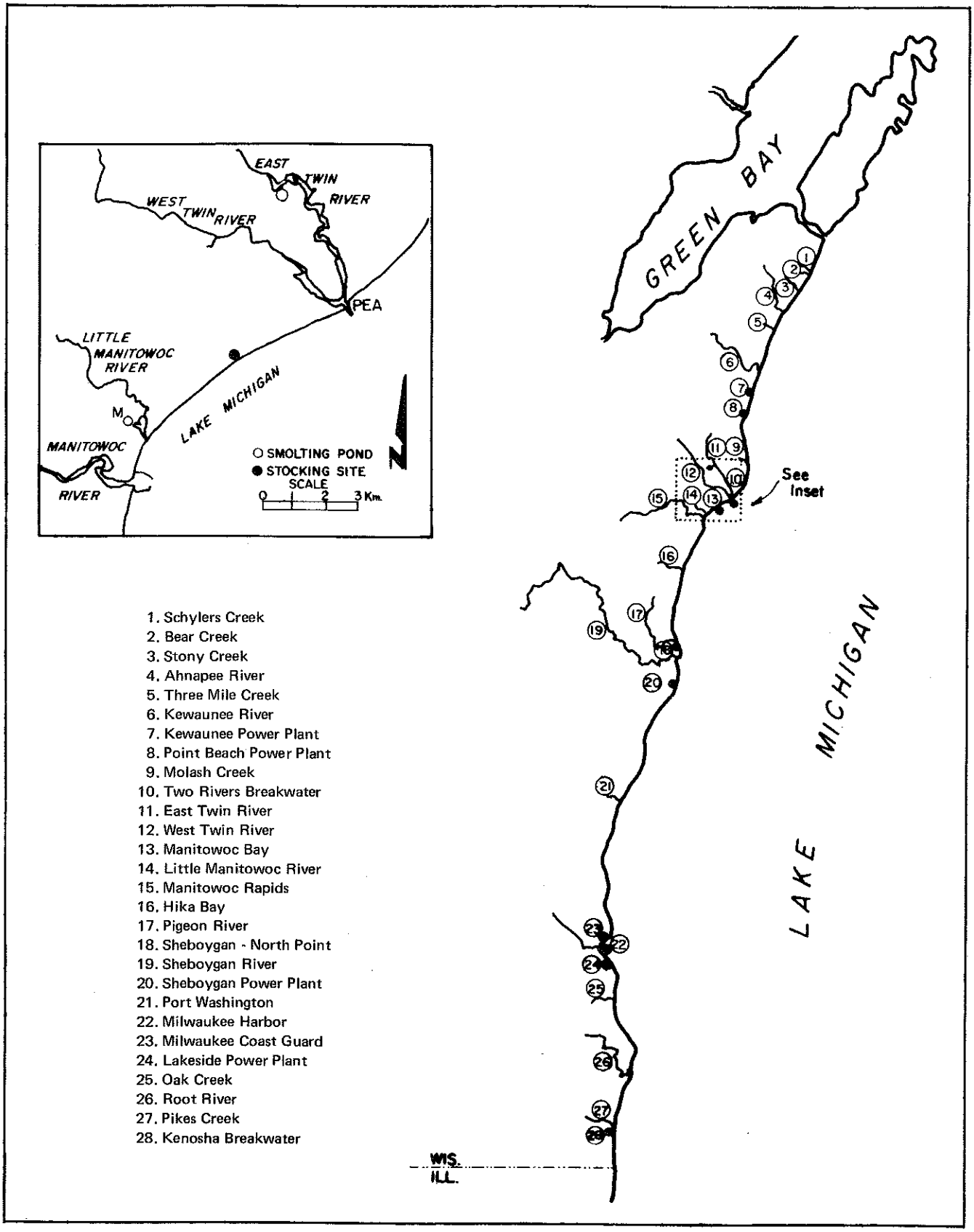


FIGURE 4. Lake Michigan shoreline indicating stations monitored during the fall of 1974. Inset map details the study area at Manitowoc and Two Rivers, Wisconsin. Morpholine was metered into the Little Manitowoc River and PEA was introduced at the Two Rivers breakwater.

Table 3. Recoveries of morpholine and PEA exposed coho salmon (O. kisutch) at individual locations. Monitoring effort is represented in terms of number of trips. Number of fish recovered is the actual number of fish observed and is not normalized for effort. Stocking location is italicized and location where PEA or morpholine was introduced is shaded gray (locations #10 and 14 respectively).

Recovery Location	Monitoring Effort		Fall 1974 No. Fish Recovered From Each Exposure Group		
	Electro-Shocking	Creel Census	Morpholine	PEA	Control
1. Schylers Creek	1	18			
2. Bear Creek	6	18			
3. Stony Creek	6	54	1		4
4. Ahnapee River	5	138		2	7
5. Three Mile Creek	5	27	2	1	1
6. Kewaunee River	5	71			2
7. Kewaunee Power Plant	0	65	1		3
8. Point Beach Power Plt.	0	58			1
9. Molash Creek	0	8			2
10. Two Rivers Breakwater	1	184	3	118	15
11. East Twin River	8	86	2	14	6
12. West Twin River	1	37		1	7
13. *Manitowoc Bay*		90	1		7
14. Little Manitowoc River	8	189	207	6	24
15. Manitowoc Rapids	5	44	2	3	31
16. Hika	-	22			3
17. Pigeon River	-	23			
18. Sheboygan - North Pt.	-	27			
19. Sheboygan River	1	44	1		3
20. Sheboygan Power Plant	-	4			
21. Port Washington	-	38			
22. Milwaukee Harbor	-	21			
23. Milwaukee Coast Guard	-	17			
24. Lakeside Power Plant	-	17			
25. Oak Creek	5	306		1	7
26. Root River	-	11			1
27. Pikes Creek	-	5			
28. Kenosha Breakwater	-	9			

Table 4. Summary of results of morpholine-PEA experiments with coho salmon (O. kisutch) at Manitowoc/Two Rivers in 1973.

Experimental Group	No. Released May 1973	No. Recovered (Fall 1974) Manitowoc (Morpholine)	Two Rivers (PEA)	Other Locations	Total No. Recovered
Morpholine	5,000	207 (94.1%)*	5 (2.3%)	8 (3.6%)	220 (100%)
PEA	5,000	6 (4.1%)	132 (90.5%)	8 (5.4%)	146 (100%)
Control	5,000	24 (19.4%)	21 (16.9%)	79 (63.7%)	124 (100%)

*(%) = Percentage of total number of fish recovered in a given experimental group and not the precentage of fish stocked.

In contrast, recoveries of unexposed fish indicated that there was considerable straying in this group. Twenty-four of these fish were taken from the Little Manitowoc River, 21 from Two Rivers and 79 from other locations. It is clear, therefore, that morpholine and PEA-exposed fish utilized chemical information for homing and could be attracted to streams or to specific areas scented with the appropriate odor.

Ultrasonic Tracking Experiments

Procedures

To supplement the results of the imprinting studies, behavioral experiments were also conducted (Madison et al. 1973; Scholz et al. 1974). These experiments were conducted at Oak Creek in 1971, 1972 and 1973. Imprinted fish were released along the shoreline of Lake Michigan north of Oak Creek and tracked into an area scented with morpholine. Control experiments were conducted by tracking fish through the same area when morpholine was not present.

Fish used for this study were captured in Oak Creek as part of the census experiments and displaced back into Lake Michigan. The fish were transported by boat to the release point located about 3.2 km north of Oak Creek along the shore of Lake Michigan (Fig. 5). An ultrasonic transmitter was inserted down the esophagus into the stomach of the fish (Fig. 6a). A directional hydrophone connected to receiving equipment on a tracking boat was used to follow the signal from the tagged fish (Fig. 6b). Positions were determined in relation to markers placed at 100 m intervals along the shoreline and the tracks were plotted on a map (Fig. 7). For details of tracking methods, see Madison et al. 1972, 1973 and Scholz et al. 1974.

The release site was selected because it was assumed that fish released along the shoreline would follow the shoreline back to Oak Creek, and thereby bring the fish into contact with the scented area (Fig. 6c). Morpholine was dripped into the lake in a line extending from the mouth of a small stream to about 100 m offshore creating an "odor barrier" through which the fish had to swim. Water currents were measured with drogues (Terhune 1968) to determine how long the chemical remained in the scented area.

Results

Fifty-six fish were tracked past the decoy area. Movement patterns between the release point and the scented area were similar for all fish (Fig. 7). In most cases, fish remained in the release area for about one hour before moving. This was possibly some sort of adjustment period resulting from handling and transport. After the fish began to move they usually travelled at a constant rate of speed without changing direction. Migration was usually along the shoreline, typically within 50 m of shore. After imprinted fish encountered the band of morpholine (Fig. 7a) they stopped migrating and remained in the scented area from 1 to 4 hours. The length of time fish remained in the area was roughly correlated with the amount of time it took for water currents to dissipate the chemical. In all cases (20 tracks) imprinted fish stopped migration when morpholine was present in the decoy area. When no odor was present (Fig. 7b), imprinted fish moved through the same area without stopping (13 tracks). Results from this experiment demonstrated that morpholine caused imprinted fish to stop migrating, although it was not clear if this behavior resulted because they were imprinted to morpholine.

It was also possible that fish reacted to morpholine because it was not the same as Lake Michigan water and thus, the behavior was not necessarily related to long term memory of morpholine. To test for this possibility, nonimprinted fish were tracked through the area when morpholine was present (Fig. 7c). Fourteen fish moved through the morpholine scented area without stopping. In addition, morpholine imprinted fish were tracked through the area when a different chemical was added and none of these fish stopped their migration (Fig. 7d). Chemicals used were N-ß-hydroxyethyl-morpholine (7 tracks) and PEA (2 tracks). Thus, it does not appear that imprinted fish were reacting to morpholine because it was a unique shoreline odor. These results indicate that the response of morpholine imprinted fish to morpholine was related to chemical (odor) imprinting and long-term memory.

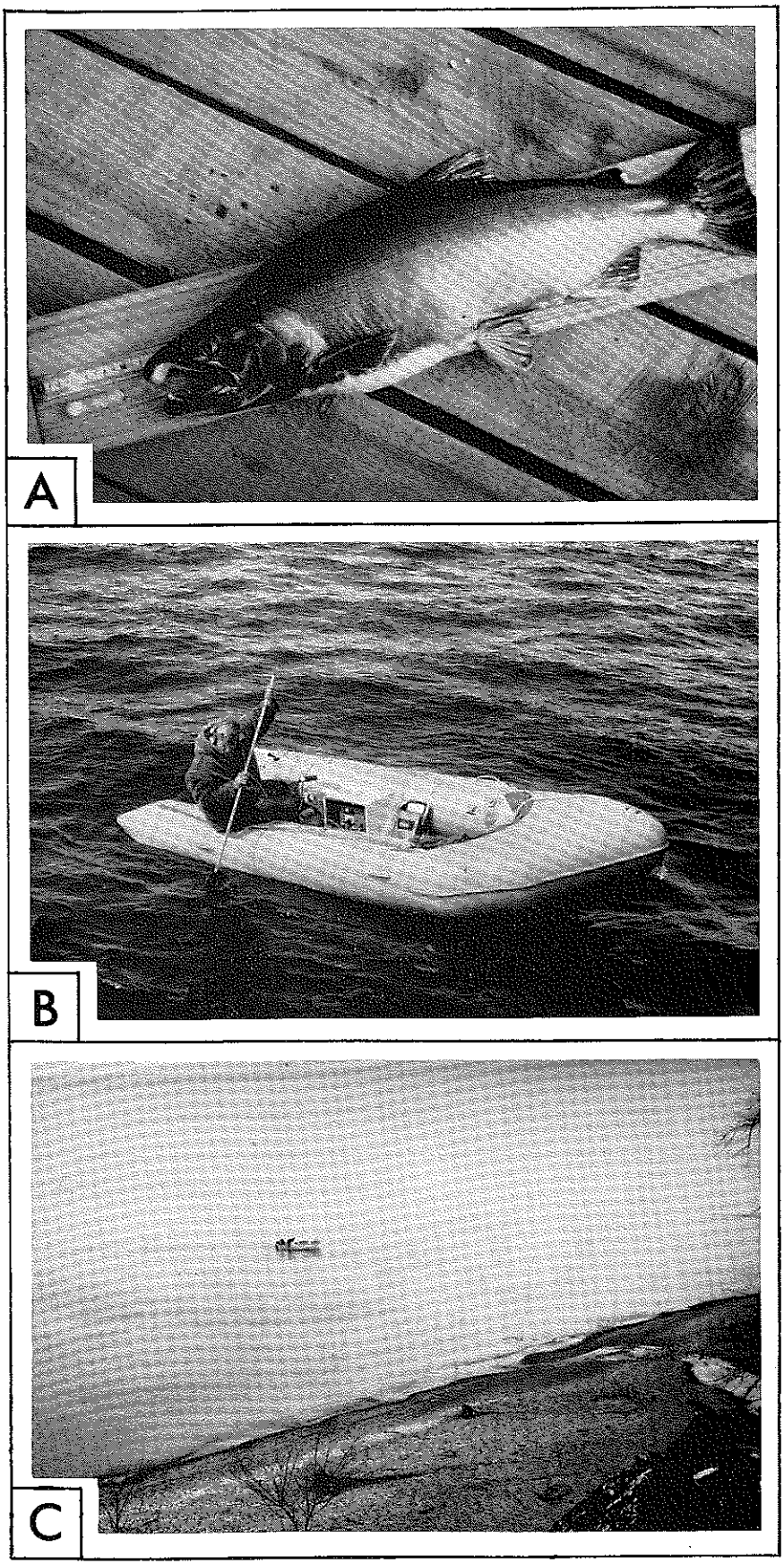


FIGURE 6. (a) Ultrasonic transmitter (lower left) shown with typical fish used for tracking experiments. (b) Ultrasonic tracking equipment: a cone-shaped directional hydrophone connected to a receiver on a tracking boat. (c) Decoy area indicating the odor barrier represented by a dye line (lower left of photo, in front of tracking boat).

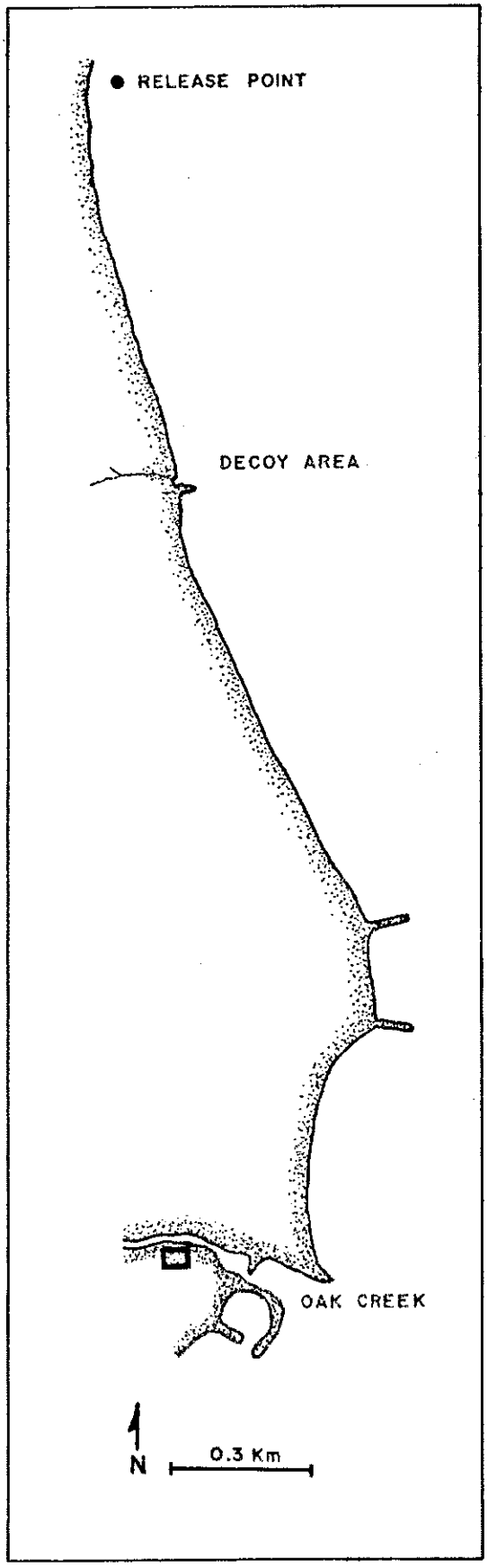
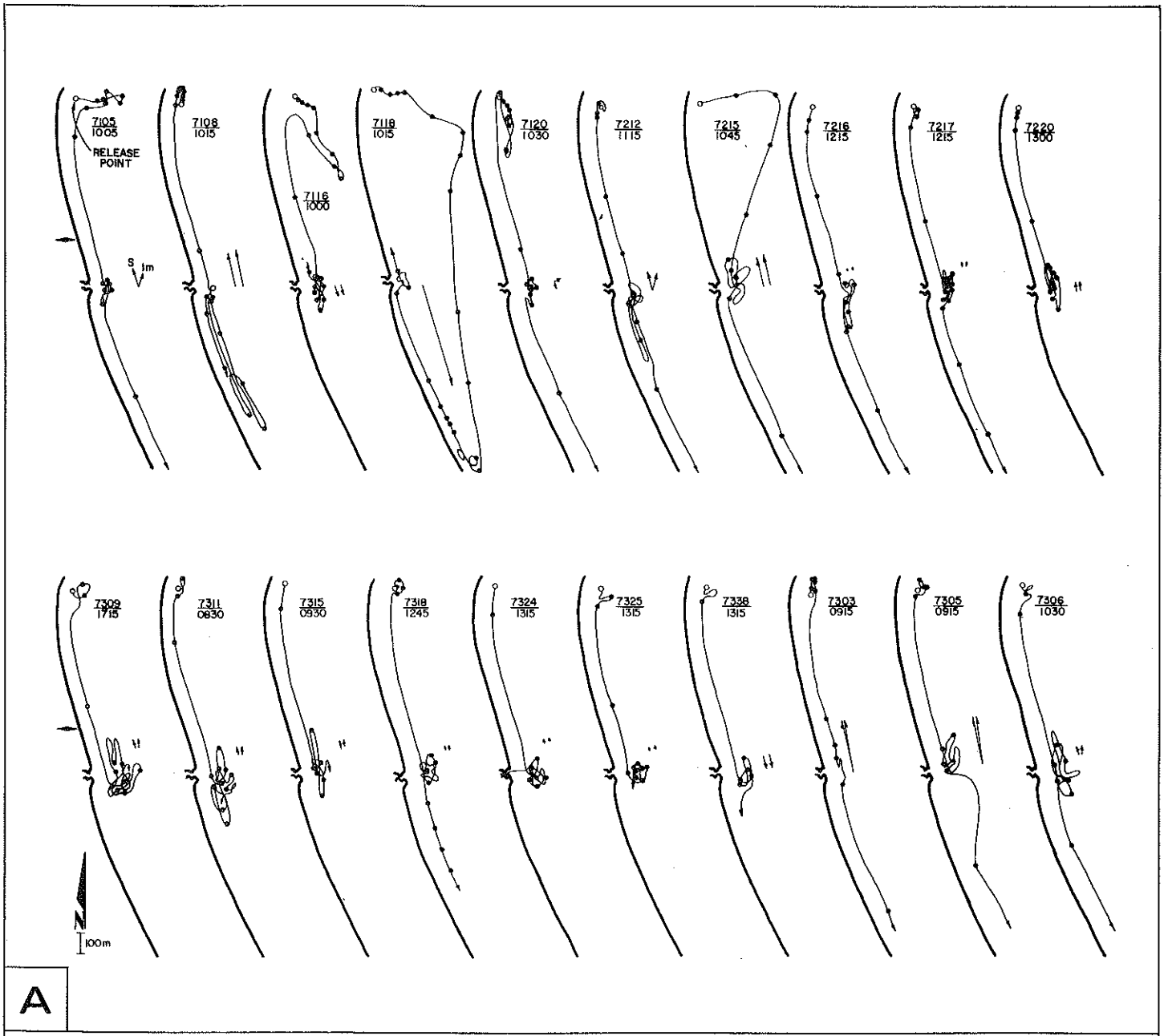
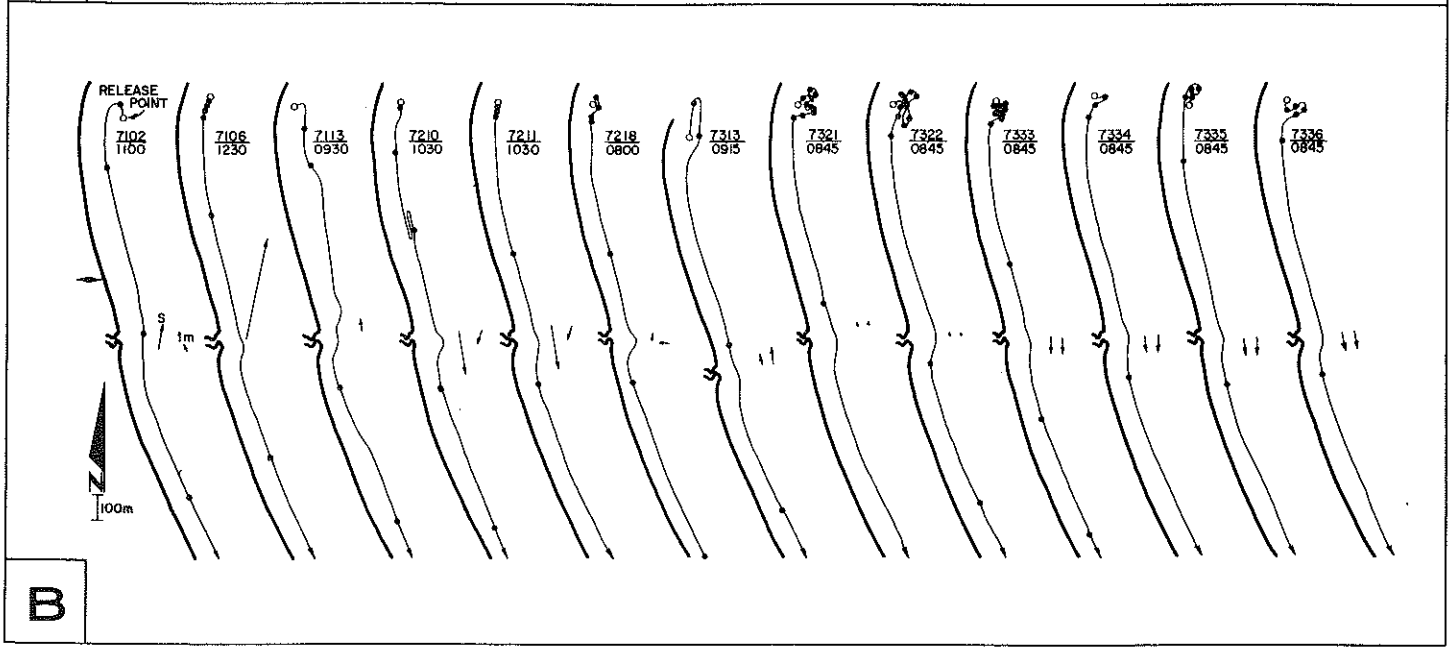


FIGURE 5. Detail of study area for tracking experiments.


7105
1005
RELEASE POINT
7108
1015
7116
1000
7118
1015
7120
1030
7212
1115
7215
1045
7216
1215
7217
1215
7220
1300
7309
1715
7311
0830
7315
0930
7318
1245
7324
1315
7325
1315
7338
1315
7303
0915
7305
0915
7306
1030
N
100m
A


RELEASE POINT
7102
1100
7106
1230
7113
0930
7210
1030
7211
1030
7218
0800
7313
0915
7321
0845
7322
0845
7333
0845
7334
0845
7335
0845
7336
0845
N
100m
B

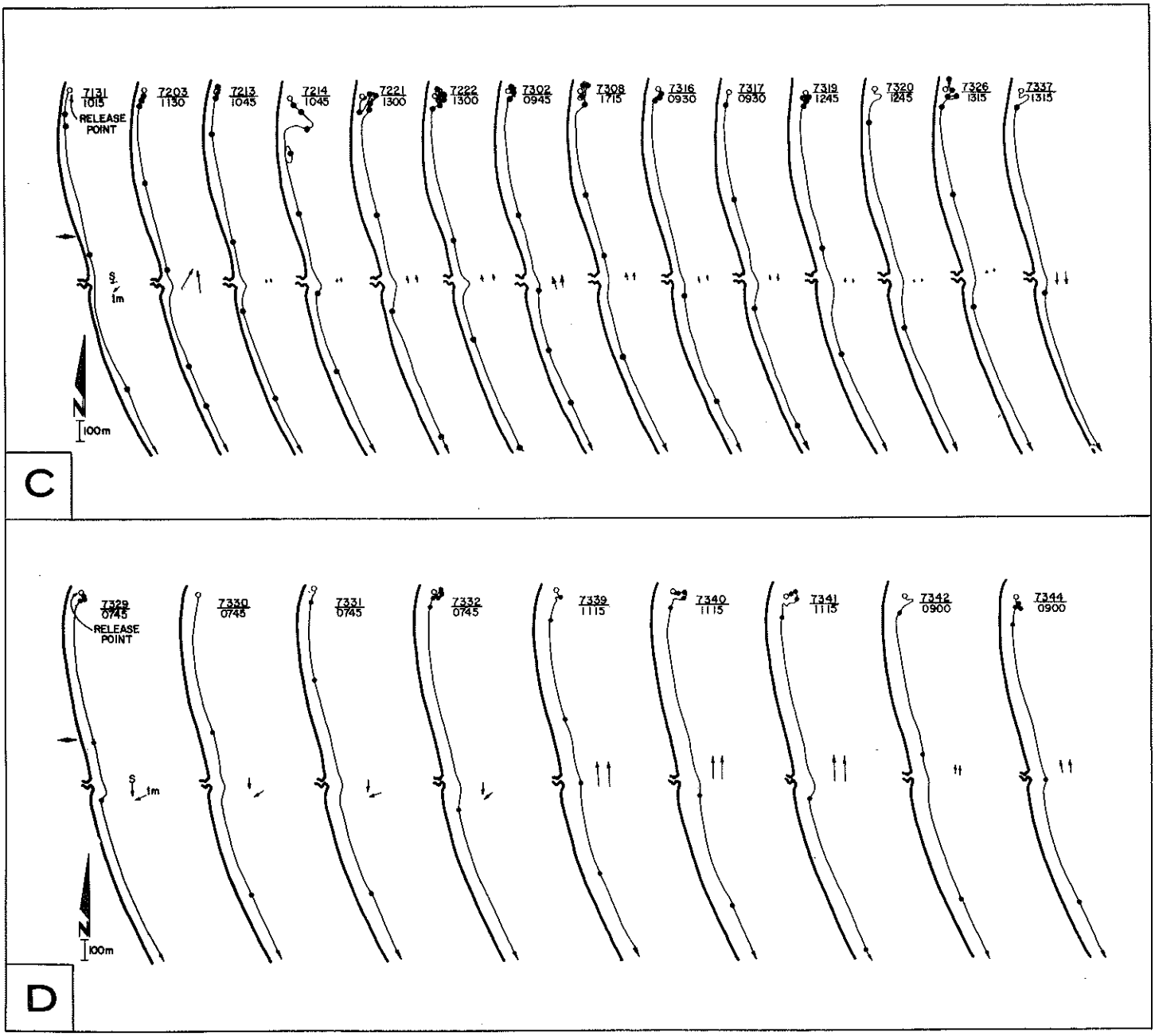


FIGURE 7. Tracks of all salmon which moved south into decoy area during behavioral experiments in 1971-73. The first two digits of each track number identify the year during which the track was recorded. Time when the track was started is recorded immediately below the track number; dots along the track path represent 15 minute intervals. Morpholine or an alternate chemical was released in the decoy area when the fish had moved to the position of the ◄►. Tracks show responses of (a) imprinted salmon when morpholine was present in decoy area, (b) imprinted salmon when morpholine was absent in decoy area, (c) nonimprinted salmon when morpholine was present in decoy area, (d) imprinted salmon when n–β–hydroxyethyl-morpholine or PEA was present in decoy area. Arrows beside decoy area represent one hour current vectors for surface and one meter currents. Imprinted fish remained in scented area for longer periods when currents were slow.

Electroencephalographic (EEG) Experiments

Procedures

In addition to the tracking experiments, electrophysiological studies were conducted at Oak Creek to determine if imprinted fish could discriminate morpholine (Cooper and Hasler 1973, 1974, 1975; MS; Scholz et al. 1974). In these experiments, olfactory bulb electroencephalographic (EEG) responses of imprinted and nonimprinted salmon to morpholine and other chemicals were recorded when chemicals were put into their nasal sac. The main purpose of these experiments was to determine if there was a difference in the response of imprinted fish and nonimprinted fish to morpholine.

Imprinted and nonimprinted salmon were captured in Oak Creek, transported to a laboratory, anesthetized and restrained in an operating box. Water was flushed through the gills to keep the fish alive. A portion of the skull over the forebrain was removed and an electrode was inserted into the olfactory bulb (Fig. 8a). A polygraph was used to record EEG signals (Fig. 8b). Dilute morpholine and a variety of other solutions, including Oak Creek water with and without morpholine, PEA and N-β-hydroxyethyl-morpholine were tested to determine if fish could discriminate morpholine from other chemicals. (See Cooper and Hasler 1973, 1974, 1975 for more details.)

Results

A total of 50 imprinted fish and 40 nonimprinted fish which returned to Oak Creek and were recorded in the census experiments were tested in the fall of 1972 and 1973.

The responses of imprinted and nonimprinted fish to morpholine were different. Morpholine-exposed fish usually responded strongly to morpholine (Fig. 9a); unexposed fish did not (Fig. 9b). In addition, imprinted fish responded to Oak Creek water plus morpholine (Fig. 9c) but did not respond to Oak Creek water alone (Fig. 9d). Other chemicals typically produced no response (Fig. 9e, 9f). These studies have demonstrated that imprinted salmon can discriminate morpholine. Dizon et al. (1973) conducted similar experiments and also determined that imprinted fish could discriminate morpholine when tested with a variety of natural waters and synthetic chemicals.

OPEN WATER MIGRATIONS

The studies described thus far have determined that salmon utilize an odor imprinting mechanism to locate a simulated home stream during the stream phase of the spawning migration. Procedures used took into account the concept that fish use other sensory systems to return from distant places in the open water of the lake to shore areas near the home stream. It was speculated that smolts might learn the location of this area at the time they were stocked. For this reason imprinted fish were released near the stream which was to be scented during the spawning migration.

To determine how close salmon had to be stocked to the simulated home stream in order to be attracted to it during the spawning migration, a series of experiments were conducted on coho salmon. Both field census and ultrasonic tracking techniques were used in these experiments.

Field Census Investigations

Procedures

These studies utilized basic imprinting methods. Fish were exposed to morpholine at a fish hatchery. The difference was in the manipulation of the stocking site and simulated home stream. Paired groups of salmon were stocked at one location and during the spawning migration the chemical was introduced at a different location. No chemicals were added at the original stocking sites. Since these experiments were conducted in different study areas, detailed information about procedures for individual experiments will be described in the results.

Results - Oak Creek

In the spring of 1972, an experiment was conducted at Oak Creek concurrently with a study previously described (see results, Table 1).

Eight thousand two hundred fish were exposed to morpholine and another 10,000 were not exposed. Instead of being stocked at Oak Creek, the smolts were released at an alternate stocking location 13 km north of Oak Creek (Fig. 2). During the spawning migration morpholine was added to Oak Creek.

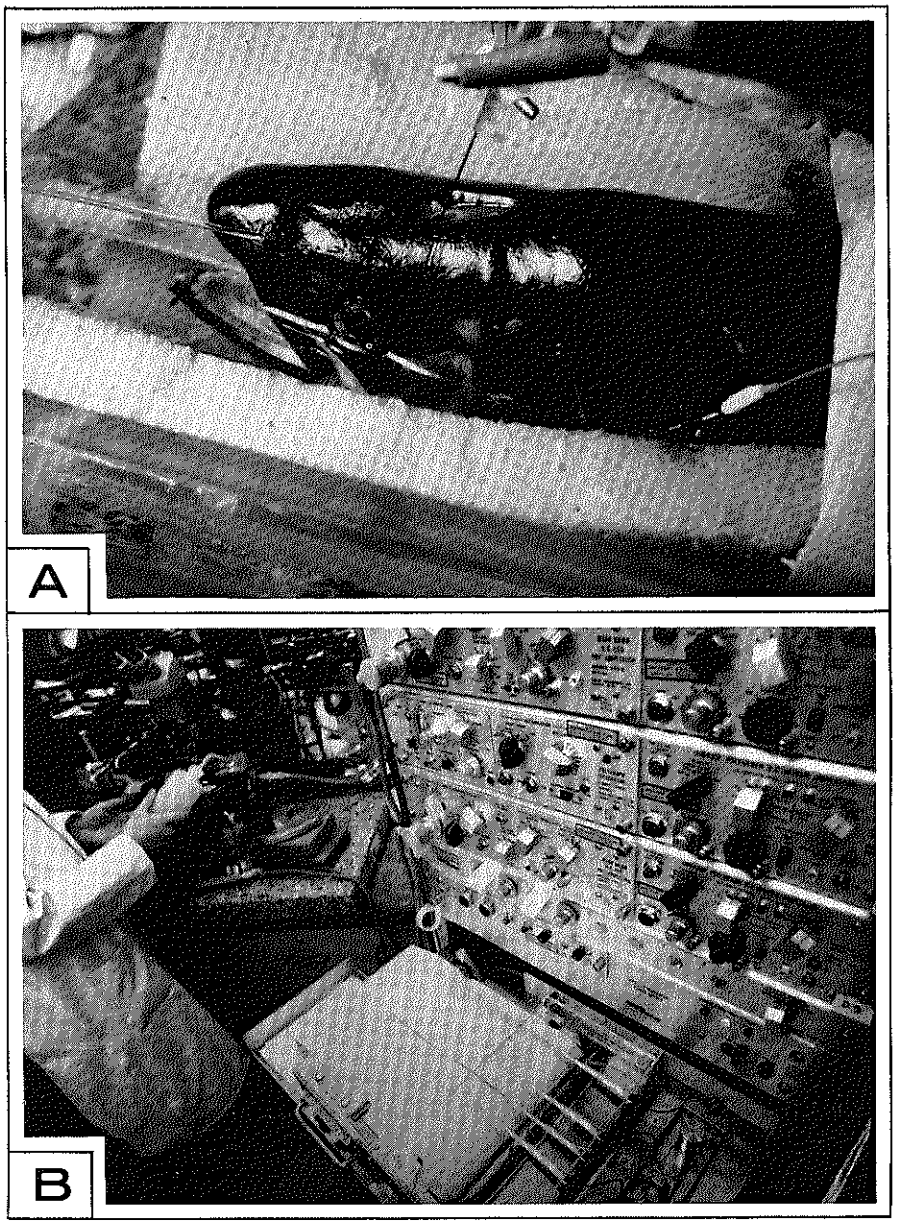


FIGURE 8. EEG recording equipment: (a) Fish in operating box with electrode inserted into hole in skull. (b) Polygraph recorder.

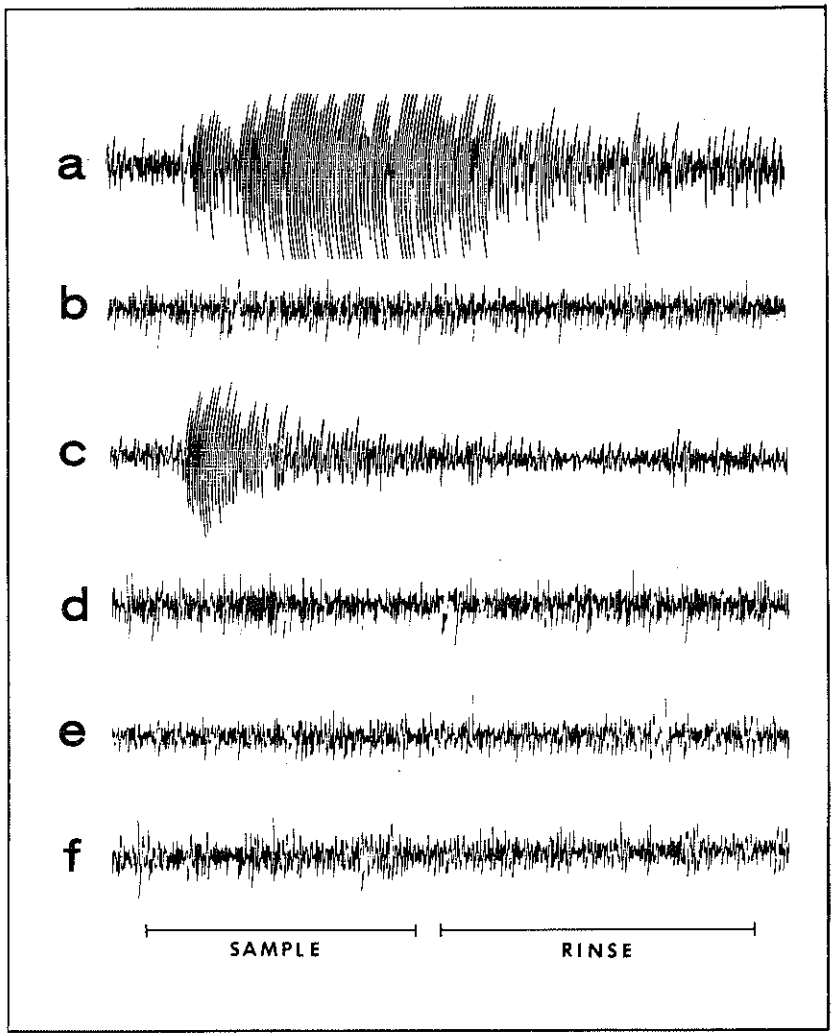


FIGURE 9. EEG responses of (a) imprinted salmon to morpholine, (b) nonimprinted salmon to morpholine, (c) imprinted salmon to Oak Creek water containing morpholine, (d) imprinted salmon to Oak Creek water alone (e) imprinted salmon to phenethyl alcohol (PEA), (f) imprinted salmon to n−β−hydroxyethyl-morpholine.

Table 5. Results of artificial imprinting experiments with coho salmon (O. kisutch) released 13 km north of Oak Creek, 1972.

Experimental Group	Fin Clip*	Number Released	Date	Number Recovered	Date	Percent of Fish Stocked
Exposed	A+RP	8,200	May 72	647	Fall 73	7.89
Controls	A+LP	10,000	May 72	65	Fall 73	0.65

*See footnote Table 1 for key to abbreviations

In the fall of 1973, 647 imprinted fish and 65 nonimprinted fish were captured at Oak Creek, a ratio of about 13:1 (Table 5), indicating that salmon can search an area of at least 13 km to locate a simulated home stream. For more details of this experiment see Cooper et al. MS.

Results - Manitowoc/Two Rivers

A similar experiment was conducted at Manitowoc in the spring of 1973. Four groups of 5,000 fish each were exposed to morpholine and four more groups (5,000 each) of control fish were not exposed. The fish were released in Lake Michigan at four locations: (1) Oak Creek, (2) Little Manitowoc River; (3) halfway between Manitowoc and Two Rivers, about 4.8 km north of the Little Manitowoc River; and (4) Bear Creek (Fig. 4). Results from the Oak creek release were previously discussed in the upstream migration section (Table 2) and fish released between Manitowoc and Two Rivers were the same fish used for the morpholine-PEA experiments (Table 4). One group of experimental and one group of control fish were released at each location. In the fall of 1974, morpholine was introduced only into the Little Manitowoc River (Fig. 4) and 28 stations were monitored.

The results from this experiment are tabulated in Table 6. Two hundred and two of the imprinted fish originally stocked near the Little Manitowoc River were captured there compared to 30 fish from the control group. Two hundred and seven imprinted fish and 24 nonimprinted fish originally stocked 4.8 km north also returned to the morpholine-scented stream. The amount of straying by imprinted and control fish was also documented. Twenty-one Manitowoc fish and 13 Two Rivers fish were recovered at other locations. Large numbers of control fish from both stocking sites returned to other streams and most of these were recovered within a radius of 48 km of their stocking locations.

In contrast, imprinted Bear Creek and Oak Creek fish were not recaptured at the Little Manitowoc River in large numbers (9 from Bear Creek and 7 from Oak Creek). Most of these fish were recovered in streams near their release area. For the Bear Creek stocked groups, a ratio of about 1:1 experimental to control fish were recovered in several of the small streams near Bear Creek. For example, 24 experimentals and 25 controls were found in Bear Creek and 34 imprinted fish and 37 controls were taken in Stony Creek.

Similar results were obtained from the fish stocked at Oak Creek. Fifty-one imprinted fish and 55 controls were recovered at Oak Creek and 4 and 6 respectively were recovered in the Root River. These results are similar to the results obtained from the control groups at Manitowc and Two Rivers, that is fish "strayed" into several streams within a radius of about 48 km of their original stocking location.

Ultrasonic Tracking Experiments

Procedures

To clarify the results of the field census experiments, behavioral experiments utilizing displacement and tracking techniques were conducted at Manitowoc. Morpholine exposed and unexposed Bear Creek stocked fish were recovered at Bear Creek and transported by truck to Manitowoc (Fig. 4). The fish were then equipped with an ultrasonic transmitter and released along the shoreline. The release point was located about 1.5 km south of the Little Manitowoc

Table 6. Recoveries of morpholine exposed and unexposed coho salmon (O. kisutch) released at four locations. Monitoring effort is represented in terms of number of trips. Number of fish recovered is the actual number of fish observed and is not normalized for effort. Stocking locations are italicized and location where morpholine was added is shaded gray.

Recovery Location	Monitoring Effort		Fall 1974 No. Fish Recovered from Each Release Group							
	Electro-Shocking	Creel Census	Bear Creek		Two Rivers		Manitowoc		Oak Creek	
			M*	C+	M	C	M	C	M	C
1. Schylers Creek	1	18	2	2						
2. *Bear Creek*	6	18	24	25				1		
3. Stony Creek	6	54	34	37	1	4		2		
4. Ahnapee River	5	138	14	16		7	8	61		
5. Three Mile Creek	5	27	13	13	2	1		1		
6. Kewaunee River	5	71	2	2		2		2		
7. Kewaunee Power Plant	-	65			1	3		3		
8. Pt. Beach Power Plant	-	58				1		1		
9. Molash Creek	-	8				2		2		
10. Two Rivers Breakwater	1	184			3	15	2	13		2
11. East Twin River	8	86			2	6	3	19		
12. West Twin River	1	37				7		1		
13. *Manitowoc Bay*		90			1	7		2		
14. *Lt. Manitowoc River*	8	189	9	1	207	24	202	30	7	
15. Manitowoc Rapids	5	44	1	3	2	31	3	30		
16. Hika Bay	-	25				1		1		
17. Pigeon River	-	23						1		
18. Sheboygan - North Pt.	-	27						1		
19. Sheboygan River	1	44			1	3		3		
20. Sheboygan Power Plant	-	4								1
21. Port Washington	-	38								1
22. Milwaukee Harbor	-	21							5	5
23. Milwaukee Coast Guard	-	17							2	4
24. Lakeside Power Plant	5	23							5	5
25. *Oak Creek*	-	306				7	5	33	51	55
26. Root River	-	11				1			4	6
27. Pikes Creek	-	5								
28. Kenosha Breakwater	-	9							3	3

* Morpholine
\+ Control

River, so that if the fish migrated in a northward direction, the correct direction to return to their stocking location, then they would come into contact with the morpholine-scented stream. Water currents in the lake were measured and the current flow at the mouth of Little Manitowoc River was monitored when the fish were near it. This was done because Lake Michigan tributary streams are affected by periodic seiche currents that can reverse their flow.

Results

Eight imprinted and 6 control fish were tracked in 1974. Imprinted and nonimprinted fish responded differently when they encountered the mouth of the Little Manitowoc River. All of the control fish and one imprinted fish moved past the mouth of the river (Fig. 10a, 10b track no. 7406). Seven imprinted fish were attracted to the mouth of the Little Manitowoc River (Fig. 10b) and 4 of them (#7401, 7405, 7409, 7410) moved into the river. During the period the fish were in the vicinity of the Little Manitowoc River, water was flowing out of the river mouth except for tracks #7413 and 7414 when the current was reversing. These two fish did not remain in the area as long as the other imprinted fish and did not enter the river. Eventually, after periods of 1 to 4 hours, 6 fish continued north. The seventh fish (track no. 7401) was captured by a fisherman.

These tracks indicated that although imprinted fish appeared to detect morpholine at the Little Manitowoc River (as judged

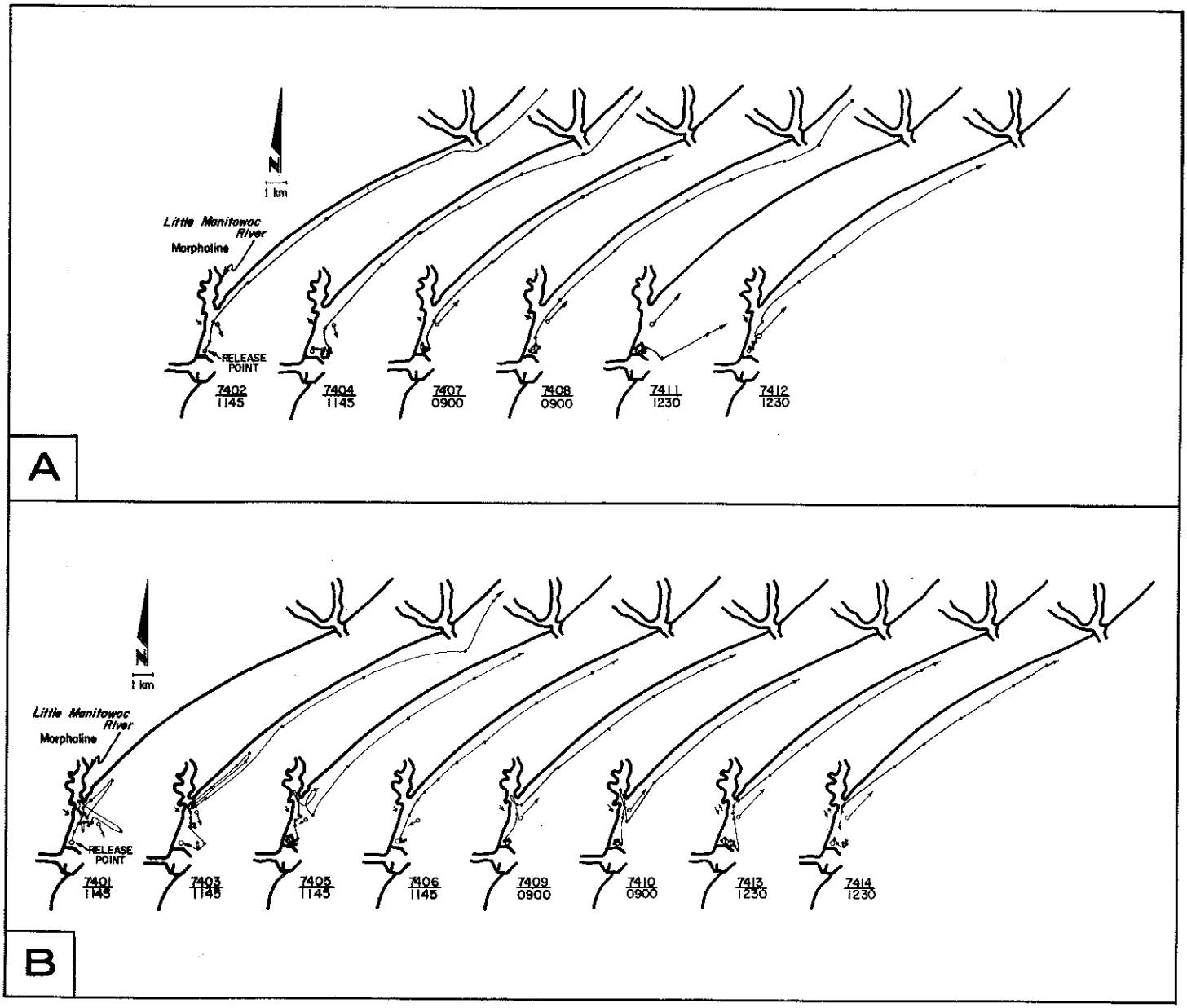


FIGURE 10. Track plots of (a) nonimprinted fish and (b) morpholine imprinted fish from Bear Creek tracked near the Little Manitowoc River. The track number is recorded above the line at the bottom of each track and the time the track was started is recorded below the line. Dots along the track path represent 15 minute intervals. Arrows beside the mouth of the Little Manitowoc River represent the direction of current flow in the river at the time the fish moved past the river mouth. Arrows in the lake are one hour current vectors for one meter currents.

by their different responses from nonimprinted fish), they do not remain at a stream treated with morpholine if that stream is 64 kilometers from the location where the fish were stocked. These results imply that fish return to a generalized region near the location where they were stocked before they will remain at a stream scented with an imprinting chemical.

SUMMARY AND CONCLUSIONS

In summary, adult coho salmon that have been exposed to morpholine (or PEA) during the smolting period, will migrate during the spawning season to an unfamiliar stream if it is scented with morpholine (or PEA). Behavioral (ultrasonic tracking) and physiological (EEG) experiments with homing adult fish have established that morpholine-exposed fish can detect and discriminate morpholine while unexposed control fish did not react to the chemical. These results confirm the existence of chemical imprinting in salmon which occurs just prior to or at the beginning of the smolting period and indicate that salmon utilize this mechanism to relocate the home stream during the spawning migration.

Coho salmon could be stocked up to 13 km from a simulated home stream and still return there to spawn. Fish stocked 64 km from the scented stream did not return there. Ultrasonic tracking experiments suggest that imprinted fish released 64 km from a scented stream do not return to the stream even if they come into contact with it. Recoveries of control fish from all experiments (and imprinted fish released a long distance from a simulated home stream) indicate that fish stray into streams within about a 48 km radius of their original stocking location. Carlin (1968) also determined that Atlantic salmon (Salmo salar) strayed within about a 40 km radius of their release site. This area may be equivalent to the area which a fish can search and find a simulated stream; therefore, fish should be stocked within about 48 km of a stream which is to be treated with an imprinting chemical.

We interpret these results to mean that there are two phases to the spawning migration of coho salmon in Lake Michigan. During the first phase fish return to a generalized region within about 48 km of the location where they were originally stocked. It appears (from the tracking experiments) that the fish must return to this region before they will enter a specific stream. It is suggested that after the fish arrive in this area they will search for a stream scented with the appropriate odor. For example, fish released 13 km north of Oak Creek were apparently able to search an area of 13 km to return to Oak Creek.

PART II. MANAGEMENT APPLICATIONS

The experiments described in Part I and in the appendix have established that coho salmon and steelhead trout can be imprinted to a synthetic chemical. These studies have determined that it is possible, in part, to direct the final stages of the spawning migration of some species of salmonids by artificially imprinting young fish to synthetic chemicals. They have also indicated that it should be possible to apply this technique in the management of salmonid populations in Lake Michigan, on the West Coast and in other parts of the world.

In terms of Lake Michigan management procedures, there are some advantages of utilizing hatchery facilities instead of smolting ponds for imprinting fish. Basically, the ponds are costly to operate compared to holding fish at a hatchery through the smolting period. For example, wells sometimes have to be drilled for smolting ponds and water pumped to supplement the natural water supply.

A second advantage of holding fish in the hatchery is that they can be observed more frequently, more easily treated for diseases, and fed more uniformly than fish held in smolting ponds. In addition, the quality of the water at the hatchery is probably better. In most hatcheries, for example, spring or well water is used to supply the raceways. Hatchery water usually remains cold throughout the entire year whereas the source of pond water is often a tributary stream which is subject to large temperature

fluctuations. In some instances, fish have had to be released from ponds before they had completed smolting because the water temperature became too warm.

In addition, it may be advantageous to stock fish directly into the lake rather than into a pond-stream system because if the downstream migration out of smolting ponds (with its associated problems of high water temperatures, disease and predation) could be eliminated, it may be possible to increase the survivial of young fish.

Another advantage of artificial imprinting is that fish can be released in areas (for example, metropolitan areas such as South Milwaukee) which do not have adequate facilities for smolting ponds.

It may also be possible that fish can be attracted or decoyed to fishing piers, fishing buoys, breakwaters or shoreline parks by dripping in the synthetic chemical at these sites. This could spread out or concentrate sport fishing pressure and eliminate some of the problems such as littering which occur along some of the coho streams in Wisconsin. For example, the experiment conducted at Two Rivers when PEA was dripped into the breakwater area has indicated that fish will stop migrating in the lower part of the river, rather than move upstream out of the influence of the odor.

Since it is possible to direct fish to return to a particular area, artificial imprinting could also be used to divert fish away from potential hazards, such as power dams or areas of warmwater discharge.

Finally, it may also be advantageous to artificially imprint species which normally are not stocked in smolting ponds. For example, steelhead trout are normally released along the shoreline because streams are usually too warm during the stocking period to permit stocking in streams. As a result, the return to the location of release is usually poor (Department of Natural Resources Unpublished Reports) and there is considerable straying into other streams. The poor returns may be because the fish were not imprinted to a home stream. The experiments described in the appendix of this report have documented that imprinted steelhead will return to a simulated home stream.

On a worldwide basis artificial imprinting could be implemented in restocking programs and in attracting (or decoying) salmon to a particular location for commercial harvest. For example, artificial imprinting could be utilized for the manipulation of Columbia River salmon and steelhead stocks.

Construction of hydroelectric dams in the Columbia River system has inundated or blocked access to certain spawning areas and impeded the upstream migration to others (Fulton 1968). For example, Lewiston Dam, located about 6 km above the mouth of the Clearwater River in Idaho, was completed in 1927. Prior to 1927 several tributaries of the Clearwater supported salmon runs, but inadequate facilities at the dam prevented fish from moving upriver and, as a result, these stocks were exterminated. Fish passage was improved in 1940 and in 1972 the dam was taken out of operation so that fish were again able to move up the Clearwater. Chinook and steelhead fingerlings have been restocked in some tributaries in order to re-establish spawning runs into these streams but success has been limited.

One problem has been that it is difficult to restock some tributaries because they are inaccessible to hatchery trucks, and use of helicopters is too expensive. It is feasible that a large number of salmon could be imprinted at a hatchery and then later decoyed into a tributary to re-establish spawning runs into these streams. For example, Dworshak National Fish Hatchery located on the Clearwater River about 68 km above the mouth releases salmon smolts each year. Usually a surplus of fish return to the hatchery to spawn, that is more than enough eggs are collected to supply the hatchery demand. If these surplus fish could be decoyed into a tributary for natural spawning, a larger production of young fish could be obtained. Once the decoyed fish spawned in a tributary their offspring would then be naturally imprinted to their stream system. Subsequent generations should continue to home to this natal tributary and, thus, a new run could become established.

An alternative approach would be to utilize chemical imprinting in conjunction with spawning channels. In some areas on the Columbia River artificial channels have been built below a dam in order to provide suitable spawning beds for the fish (e.g. Priest

Rapids dam). Water is pumped from the dam into the channel. Salmon fingerlings were stocked in these spawning channels with the hope that adult fish would return there to spawn and thereby establish a spawning run.

The method has not proven very successful because fish do not re-enter the channel at the time of spawning and instead stray into areas which are not suitable for spawning (Allen and Meiken 1973). One possible reason for this may be because the odor of the water coming from the channel is identical to the river water so that the fish were not able to differentiate the entrance to the spawning channel. It should be possible to scent the channel with a synthetic chemical when the young fish are stocked and later when the adults return to spawn, to re-scent the channel so as to permit homing to occur.

PART III. IMPRINTING METHODS AND EQUIPMENT[1]

It has been shown that artificial imprinting can be a useful technique for the management of salmon fisheries. For this reason the following summary of imprinting methods has been prepared.

CHEMICALS

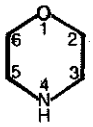
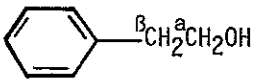
Morpholine and phenethyl alcohol have been used successfully for artificially imprinting salmon and trout. A summary of the physical and chemical properties of these chemicals are presented in Table 7. Theoretically, it should be possible to utilize almost any synthetic chemical for imprinting, providing it can be detected by the fish. However, the selection of a particular chemical may be determined by the following criteria:

1. It should not be found in natural waters. It should not be a chemical that, for example, a pulp mill or power plant continuously pumps into the water because of the possibility of decoying the fish to an unsuitable area.
2. It should be chemically stable in the natural environment.
3. It should be highly soluble in water, thereby reducing problems with mixing with large volumes of water.
4. It should be an organic compound because earlier work (Hasler and Wisby 1951; Idler et al. 1961; Cooper et al. 1974) has indicated that the identifiable component of home stream water is contained within the organic fraction.
5. At the low concentrations used for imprinting, it should be neither a generalized attractant nor a repellent which could induce all salmon to be attracted to or repelled from a stream during the spawning migration whether or not they had been exposed to the chemical during the smolting period. For example, salmon are repelled by some substances in very low concentrations. It has been shown that rinses from mammalian skin can be detected by salmon at low concentrations but behavioral experiments (Brett and Mackinnon 1954) have determined that this odor will stop salmon from migrating upstream.
6. It should be non-toxic and detectable at concentrations low enough so that only small amounts need to be added to a stream. This would reduce any possible damage the chemical might have on the natural stream system.

The level at which a fish can detect a chemical is usually determined by behavioral conditioning techniques or observations of normal behavioral responses (Wisby 1952; Walker 1967; Tarrant 1966). Ideally the level for each chemical should be tested for a particular species since the detectable level may be different for each species.

[1]Statement of brand names, model numbers or ordering addresses does not represent endorsement of the product or company by either the University of Wisconsin or the Wisconsin Department of Natural Resources.

Table 7. Summary of properties of the imprinting chemicals morpholine and phenethyl alcohol (after Dizon 1971; Merik Index 1968).

Property	Morpholine	Phenethyl Alcohol
Formula	C_4H_9NO 	$C_8H_{10}O$ 
Other Names	Tetrahydro-2H-1, 4-oxazine; tetrahydro-1, 4-oxazine; diethylene imidoxide.	2-Phenylethanol; -phenylethyl alcohol; benzyl carbinol; -hydroxyethyl-benzene.
Molecular Weight	87.12	122.16
Density (g/ml)	d_4^{20} 1.007	d_{25}^{25} 1.017 - 1.019
Solubility	Infinite in H_2O	1.6 g/100 ml H_2O at 20°C 2.0 g/100 ml H_2O at room temp. with thorough shaking.[1]
Color	Colorless liquid	Colorless liquid
Odor	Characteristic amine odor; sharp, ammoniacal.	Floral odor, rose character
Uses	Cheap solvent for resins, waxes, casein, dyes. Morpholine fatty acid salts are used as surface-active agents and emulsifiers. Other morpholine compounds are used as corrosion inhibitors, antioxidants, plasticizers, viscosity improvers, insecticides, fungicides, herbicides, local anesthetics and antiseptics.	In flavors and perfumery. Practically all rose perfumes are compounded with it. Medical Use: has been used as antibacterial agent in ophthalmic solutions.
Effects on Humans	Irritating to eyes, skin, mucous membranes. May cause liver, kidney injury.	A strong local anesthetic. Experimentally has caused severe CNS injury in mice.
Miscellaneous	Mobile, hygroscopic liquid. Volatile with steam. Strong base.	Found in a number of natural essential oils such as rose, carnation, hyacinth, Aleppo pine, orange blossom, geranium, bourbonneroli, and in the essential oil of champaca.

[1]Because of low solubility, water solutions of PEA are used for dripping only in cases of low flow rates.

In experiments described in this report, morpholine was selected for imprinting the salmon because Wisby (1952) had previously determined that it could be detected by untrained coho salmon at low concentrations (1×10^{-6} mg/l) and PEA was chosen because Teichmann (1962) reported that rainbow trout could be conditioned to respond to phenethyl alcohol at a concentration of 3.6×10^{-4} mg/l. Table 8 summarizes the results of behavioral experiments which have been conducted to test the sensitivity of different species of salmon and trout to selected chemicals.

In addition to the chemicals listed on Table 8, Wisby (1952), Brett and MacKinnon (1954) and Tarrant (1966) have tested the responses of coho salmon and chinook salmon to various chemicals at different concentrations; Walker (1967) has tested Atlantic salmon and Kleerekoper (1969) has summarized the studies conducted with both salmonids and nonsalmonid species.

Chemicals can be purchased at a chemical supply house and should be purchased well in advance of an application since they are not always in stock.

DELIVERY SYSTEM

The delivery system utilized for the experiments is diagrammed in Figure 11. Imprinting equipment consisted of flexible tubing, a pump and a 19 liter glass jug filled with distilled water and imprinting chemical. Glass was used rather than a metal, plastic or wooden container because it would not react with the chemical in the jug. Distilled water was used as a solvent for the imprinting chemical for a similar reason.

A glass tube was inserted through a cork at the top of the jug. A piece of 6.0 mm tygon tubing connected the glass tube to special thick-walled, 3.0 mm flexible pump tubing (Fig. 11a). The tygon tubing was fitted tightly over the rod and inserted onto the mating end of a syringe needle. The point of the needle was inserted into the thick-walled pump tubing and had to be blunted with a file or it would cut into the side of the tubing. Wire or string was lashed around hose ends to insure tight connections (Fig. 11a). A syringe needle was punched through the cork to allow air pressure to equalize in the jug as the solution was drained off.

A peristaltic or, alternately, a piston pump capable of pumping small quantities (on the order of 10 to 20 ml/hr) and independent of the flow of gravity was used to meter the chemical solution from the jug into the raceway or test stream. Both pumps operated on 110 VAC current.

Peristaltic pumps operate by squeezing the flexible tubing between a rotating pump head and a backplate (Fig. 11c). Silicone grease was applied in order to reduce friction between the rotating unit and the tubing. A hose clamp prevented the tubing from sliding through the pump. Flow rates could be controlled by adjusting a speed control knob. This type of pump usually has several channels, which means that more than one raceway can be serviced by one pump. Since each channel has its own tube, one pump can also be used to meter different chemicals.

The peristaltic pumps have several disadvantages. The tubing on which the rotating head bears is expensive (approx. $1.50/ft). It also breaks frequently which means that the pump must be inspected often; at least once a day is recommended. Another problem is that it is difficult to adjust the pump (i.e. to get the right amount of pressure bearing against the tubing to cause the solution to move). The hose clamp must also be adjusted carefully. It must be clamped tight enough to prevent the tubing from moving but not so tight as to prevent flow through the tubing. If the clamp is loose and the pressure on the back plate is low, the chemical will siphon out of the jug.

The peristaltic pump used for the experiments in this report was a four-channel Buchler polystatic pump Model No. 2-6100 (Buchler Instruments, 1327 16th Street, Fort Lee, New Jersey 07024). The cost is approximately $590.

Because of the problems with the peristaltic pumps, changeover was made to a piston driven pump which eliminates trouble with hose breakage since the tubing is not used in the pumping action (Fig. 11b). In addition, this pump is easier to adjust and also seems to deliver solutions at a more constant

Table 8. Detectable levels of chemicals for various species of salmonids.

Species	Chemical	Concentration(mg/l)	Reference
Coho salmon (Oncorhynchus kisutch)	Morpholine	1×10^{-6}	Wisby 1952; Hasler 1966;
Coho salmon (O. kisutch)	Morpholine	5×10^{-4} to 5×10^{-6}	Cooper and Hasler 1973, 1974; Madison et al. 1973; Scholz et al. 1974; Steffel 1972
Coho salmon (O. kisutch)	Phenethyl alcohol	1×10^{-3} to 5×10^{-3}	Madison et al. 1973; Cooper 1974
Coho salmon (O. kistuch)	Eugenol	1.8×10^{-1}	Tarrant 1966
Coho salmon (O. kisutch)	Potassium phenethyl acetate[1]	1×10^{-7}	Hasler and Wisby 1959[1]
Atlantic salmon (Salmo salar)	Morpholine	1×10^{-2}	Walker 1967
Rainbow trout (Salmo gairdneri)	Ionone	3×10^{-4}	Teichmann 1959
Rainbow trout (S. gairdneri)	Phenethyl alcohol	3.6×10^{-4}	Teichmann 1962
Rainbow trout (S. gairdneri)	Morpholine	5×10^{-4} to 5×10^{-5}	Cooper and Scholz, MS (See Appendix I)
Brown trout (Salmo trutta)	Morpholine	5×10^{-4} to 5×10^{-5}	Scholz and Cooper, in prep.

[1]This chemical is a distinct repellent and thus, probably would not be good for imprinting fish. It has been used to repel salmon during periods of construction of dams on the Columbia River.

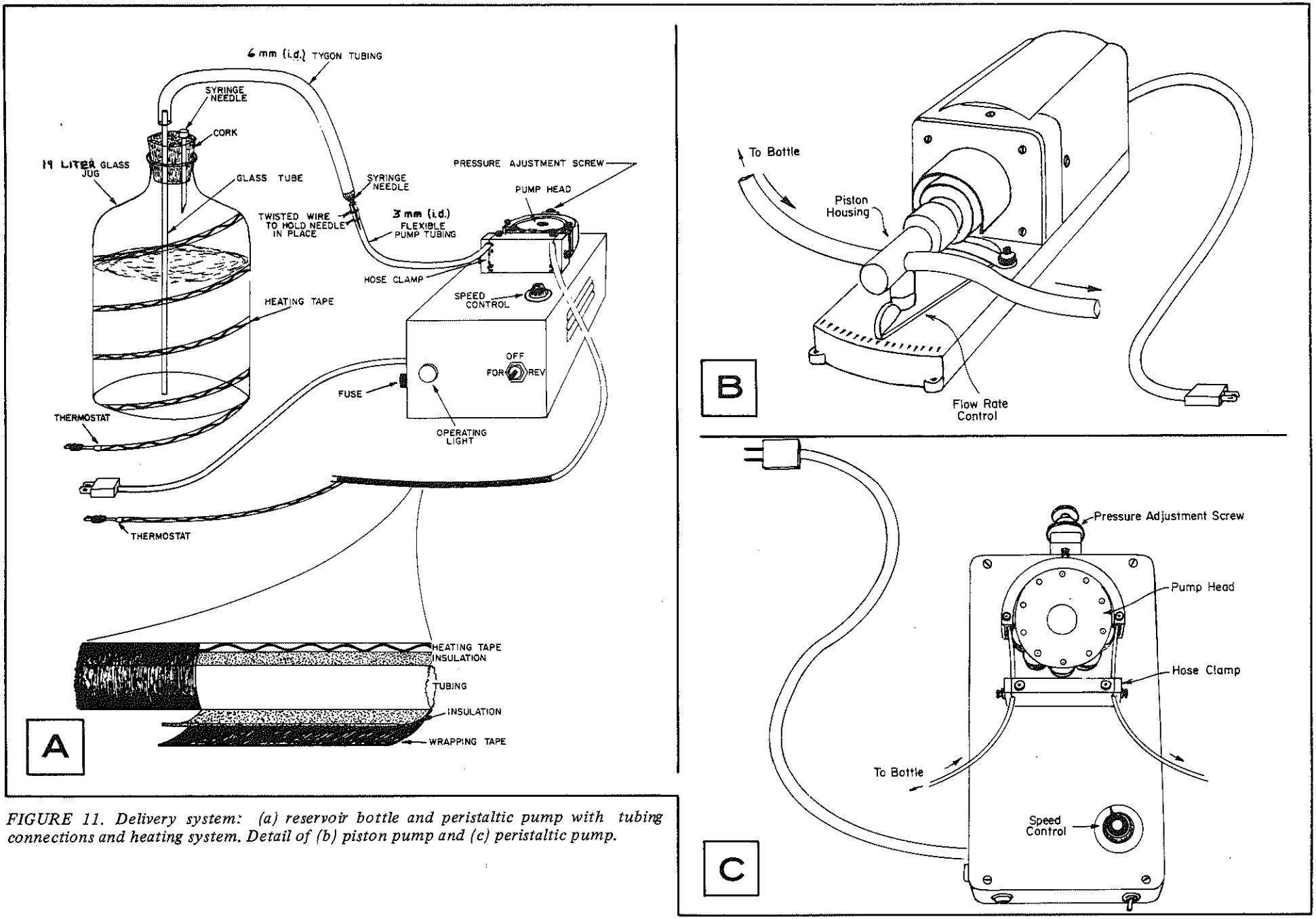


FIGURE 11. Delivery system: (a) reservoir bottle and peristaltic pump with tubing connections and heating system. Detail of (b) piston pump and (c) peristaltic pump.

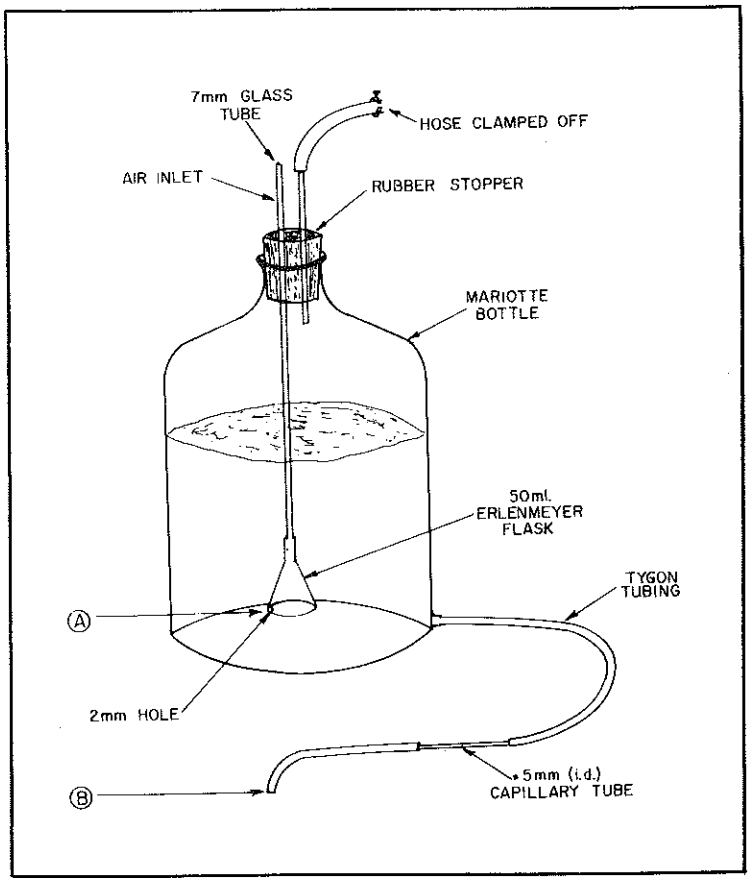


FIGURE 12. Mariotte Bottle (after Kubitschek, 1954). Provides constant low flow rates (10-20 ml/hr.) independent of atmospheric pressure (that is, independent of height of liquid in bottle). Flow rate is controlled by length and diameter of capillary tubing and by adjusting height between (a) the hole in funnel at the bottom of the air inlet tube and (b) the outlet dripper.

rate. The piston pump used for some of the experiments was Model No. RP1G, purchased for approximately $250 from Fluid Metering, Inc., 48 Summit St., Oyster Bay, NY 11771. From our experience, piston pumps appear to be more reliable than peristaltic pumps if the pump is to be left unattended for long periods. The primary disadvantage of the piston pump is that it has only one channel to deliver chemicals so only one raceway could be treated.

One disadvantage of both peristaltic and piston pumps is that they operate on 110 VAC. At a hatchery this is not a problem but it could be in remote streams which are to be scented during the spawning migration. Gravity flow drippers could be utilized in these situations but the drip rate does not remain constant (Smith et al. 1974, p. 34). However, Mariotte bottles (Kubitschek 1954) which operate on a drip system without electricity have recently been tested in Wisconsin and shown to operate as well as a peristaltic or piston pump (Fig. 12). Small electric pumps which operate off a 12 VDC battery have been utilized to meter lampricide into streams where electricity is not available (Anderson 1962; Applegate et. al. 1961). The main problem with many of these pumps is that large amounts of solution are pumped during a short period of time. Fluid Metering, Inc., however, sells a piston pump (Model RP1BG25) which operates on 12 VDC and has comparable flow rates to the 110 VAC model described in this report. The price is about $250.

HEATING SYSTEM

The jug and pump were housed in a weather-proof box (Figs. 1, 11). During cold weather it is advisable to rig a heating system to prevent freezing. Heating cables with automatic thermostats were used. The cable activated when the temperature inside the box dropped below 2°C. The cable was wrapped around the jug (Fig. 11a), and provided enough heat inside the box to keep the pump and solution in the jug from freezing. A second heating cable was extended along the line from the box to the raceway (or stream). Insulation was wrapped around the tubing and cable, and plastic tape was wrapped around

the insulation to protect it from rain and snow. A thin layer of insulation was placed between the heating cable and the tubing to prevent scorching of the tubing (Fig. 11a).

CALCULATIONS OF STREAM FLOW AND CHEMICAL CONCENTRATIONS

Concentrations need to be carefully controlled during the imprinting and decoy periods because it is not known if fish which are imprinted to a certain concentration will return to a stream which is scented with a much higher or much lower concentration than those to which they were imprinted.

The steady state concentration was calculated with the following formula (Dizon 1971):

$$DC = \frac{SSC \times FR}{DR}$$

Where: DC = Drip Concentration (g/l)
SSC = Steady State Concentration (g/l)
FR = Flow Rate (l/sec)
DR = Drip Rate (l/sec)

The drip concentration is the amount of chemical in grams which is to be added to 1 liter of water. This is what is solved for in the equation since the other three values are known or can be calculated. To calculate the drip concentration, four things need to be determined:

1. Steady state concentration of chemical in the raceway or stream. This is a known value (the level which can be detected by fish). Steady state concentrations in this paper are presented in mg/l and therefore need to be converted into g/l to fit this equation.
2. The drip rate of the metering pump. The pump should be calibrated using a graduated cylinder several times at different speeds. Since various factors will cause pumps to change calibration they should also be inspected before each use or at regular intervals if prolonged use is required. The drip rate was determined from the pump calibration. This was originally measured in ml/hr and had to be converted to l/sec.
3. Flow rate. This is the volume of water (in l/sec) flowing through a raceway or stream. A conversion is usually necessary since flow rates are normally determined in gal/min or cfs units. Most major rivers are gauged and there are state and federal publications which report daily flow rates and also maximum, minimum and mean flow for each month of the year (U.S. Geological Survey, Water Resources Data Reports for individual states). Chemical concentrations are usually calculated for mean flow if it is available. For rivers or streams which do not have gauges or for hatchery raceways, flow rates can be calculated by several methods (Corbett and others 1954). One method which seems to be accurate has been described by Vannard (1961) and Dizon (1971). This technique involves determining the amount of water flowing over a broad-crested weir:

$$TF = W\sqrt{C \cdot D^3}$$

Where:
TF is the total flow (ml/sec)
W is the width of the dam (cm)
C is the constant, the acceleration due to the gravity (980.7 cm/sec^2)
D is the depth of the water flowing over the weir (cm)

Since most hatcheries have weirs on either end of a raceway, this formula was used for calculating all flow rates in the hatcheries in the experiments described in this report. Flow rates were checked at both upstream and downstream weirs. If a dam or weir is not present, flow rates can be calculated directly by taking the cross-sectional area of a stream and multiplying it by observed current rate at several depths and locations (Corbett and others 1954; Rayner and Schmidt 1957). Urquhart (1957) has discussed how to determine flow rates more precisely.
4. The amount of chemical to be added to a jug. To determine this, the volume of the jug in liters should be multiplied by the drip concentration (in g/l). An 18.95 liter bottle was used so that the chemical solutions would last for a long time without replacement. With the pumping rate set for 10 ml/hr, a bottle lasted approximately 40 days.

At the hatchery the chemical was metered into the raceways immediately below the upstream falls to insure complete and rapid mixing of the chemical with the water.

If flow rates are measured in cfs units, Applegate et al. 1961 and Smith et al.

1974 have used the following formula to obtain the steady-state concentration so that no conversions are necessary:

$$F' = \frac{C \times F}{0.03713 \times C'}$$

Where: F' = rate of pumping solution in U.S. gallon per hour
F = volume of flow of the stream in cubic feet per second (cfs) at the point of introduction of the chemical
C' = concentration of stock solution in grams per liter
C = concentration of the chemical in mg/l desired in the stream at the point of introduction, and
0.03713 = conversion factor

Sample calculations are presented in the appendix.

WATER SOURCE (ODOR ENVIRONMENT)

If fish are to be decoyed to a location different from the imprinting location, they should be held in a "neutral water source" during the exposure period. Neutral water is considered to be water which the fish will not come into contact with during the spawning migration. By using this water instead of, for example, water from a natural stream which drained into Lake Michigan, problems that might result from mixing the artificial with the natural odors could be avoided. It is possible, for example, that the natural odor alone might cause the fish to return to the imprinting location instead of the treated stream. In situations where fish must be imprinted in a natural river system, they should be decoyed to a location downstream from the location where they were imprinted.

STOCKING LOCATION

Experiments described here have demonstrated that fish will return to the general region of their stocking location probably by nonolfactory mechanisms and then search within this region for a simulated home stream. Information to date suggests that the area which they will search appears to be about 13 to 48 km on either side of the location where they were stocked. This means that the fish should be stocked within this range of the stream which is to be treated with the chemical during the spawning migration.

EXPOSURE PERIOD

Probably the most critical factor in an imprinting program is the selection of the correct sensitive period during which the salmon must be exposed to an imprinting chemical. In our experiments salmon were exposed to an imprinting chemical during their presmolting and smolting stages.

Smolting is one of the "critical" periods in the life history of salmon when they are undergoing physiological changes (Hoar 1951; 1958) at the beginning of their downstream migration. In the experiments described in this paper, smolting occurred at about the same time every year, usually in late April or early May. Imprinting was initiated in early April and continued for about 5 or 6 weeks until about 1 to 2 weeks after the first signs of smolting were observed. In Lake Michigan, coho salmon smolting was typically characterized by loss of parr marks and changes in body coloration from dark green or black to silvery. In addition, prior to smolting, the fish appeared to be distributed randomly, scattered throughout the raceway or pond. By the time they were released, schooling was more pronounced and most of the fish were observed crowding the downstream end, especially at night.

This period was selected for imprinting because in earlier studies (notably Donaldson and Allen (1957), Carlin (1968), Jensen and Duncan (1971), Ricker (1972), Fessler (1974), Vreeland et al. (1975) and Roy Wahle (pers. comm.) salmon which were transported to a new location just prior to or at the onset of smolting returned to the location of release rather than to the "parent" stream.

For example, in April 1967, Jensen and Duncan (1971) transplanted 650,000 coho salmon which had just started to smolt from Leavenworth National Fish Hatchery on the Columbia River to a location about 258 km downstream at a spring fed fish handling facility at Ice Harbor Dam on the Snake River (Fig. 13). The smolts were held in this water for 36 to 48 hours, marked and then released. In September through

Table 9. Number of coho salmon (O. kisutch) captured by a trap at Ice Harbor Dam in relation to the type of water used to attract fish (from Jensen and Duncan, 1971).

Source of Water	Date of Capture	Number of Salmon
Spring Water (gravity flow)	November 2	52
	3	208
	10	5
	11	2
	14	7
	15	18
	16	18
	17	26
Spring Water (pumped)	November 7	59
	9	4
	TOTAL	399
River Water (pumped)	November 4	0
	5	0
	6	0
	8	0
	12	0
	13	0
	TOTAL	0

November 1967, 1,712 precocious male coho (jacks) were recaptured near the spring water discharge about 0.8 km downstream from the release location. No fish were recovered at Leavenworth Hatchery. Behavioral studies also demonstrated a clear attraction to spring water. Water from the holding facilitity was piped through a floating trap to serve as attraction water. To determine if the fish were actually homing to the water in which they had been held as smolts, river water was alternately pumped through the trap. No fish entered the trap when river water was used, but a total of 399 fish were recovered during periods when spring water was pumped through the trap (Table 9).

In this example there seems to be little doubt that the spring water from the fish holding facility was the orienting stimulus and that fish were able to learn the odor of this water within two days. In contrast, Peck (1970) released post-smolt coho into a tributary of Lake Superior and the return to that river was very poor with large numbers of these fish being recovered in other streams. This would tend to indicate that at some period of time after smolt transformation the fidelity of the imprinting process breaks down.

In another study, conducted over a period of 3 years by the National Marine Fisheries Service in Seattle, (Don Park and Wes Ebel pers. comm.) chinook salmon, steelhead trout and some coho salmon smolts were collected at Little Goose Dam on the Snake River. At this point the fish had already migrated downstream about 240 km to 320 km. The fish were marked and then transported about 320 km downstream to below Bonneville Dam on the lower Columbia River (Fig. 13). During the spawning migration a high proportion of these fish returned to the upper Snake River above Little Goose Dam, not to the area of release. There was a small degree of straying into streams near the transplant location.

Similarily, in a study reported by Ebel et al. in 1972, chinook salmon were collected at Ice Harbor Dam during their downstream migration and transported to below Bonneville Dam and released. The salmon returned as adults to Ice Harbor Dam and other locations upriver. There was no indication of straying into streams near the release area. Slatnik et al. (1975) repeated this experiment with similar findings. In Scotland, Atlantic salmon captured during the smolt migration and tranported downstream also returned to locations upriver to spawn (Mills and Shackley 1971).

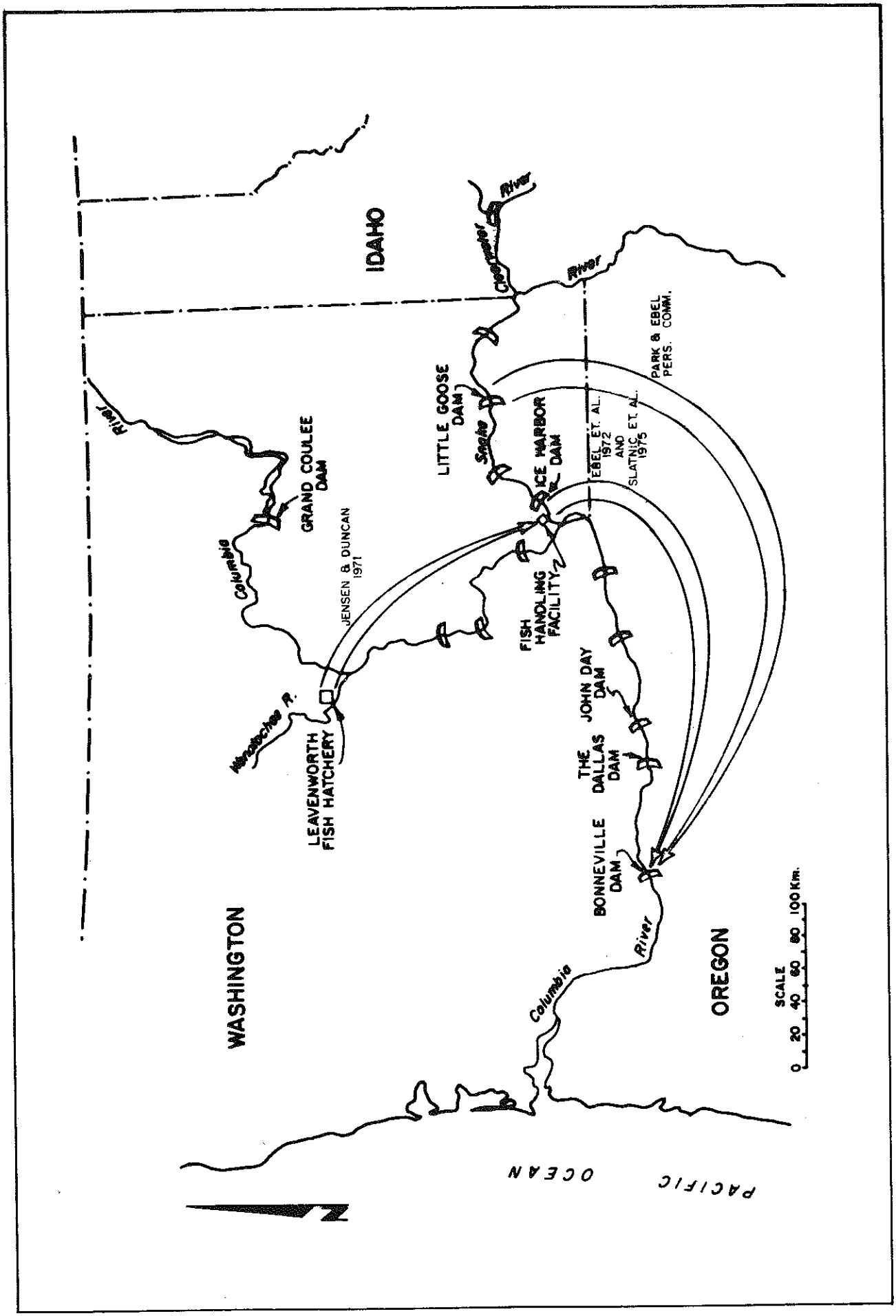


FIGURE 13. Diagram of research area used for transportation studies on the Columbia River.

On the other hand Vreeland (1975) stated that "Experiments conducted at hatcheries in Oregon, Washington, and California have shown that a majority of chinook salmon, coho salmon and steelhead trout transported and released as smolts, but with no downstream migration prior to hauling, returned to the area of release as adults." Thus, it has been suggested that imprinting would terminate soon after the beginning of downstream movements, thereby preventing fish from learning information about river areas or streams during their downstream migration (Peck 1971; Madison et al. 1973).

There is further information that the imprinting process is apparently rapid. As previously described, results of Jensen and Duncan's study (1971) indicated that fish could be imprinted within two days. Carlin (1968) also reported that Atlantic salmon held in a tributary for two days returned to that tributary to spawn. Jim Mighells (pers. comm.) has conducted preliminary experiments with transplanted fish which indicate that coho salmon will imprint to a water source within 4 to 24 hours. Cooper (1974) reported that, based on a coho jack return, fish imprinted to morpholine for two days at the onset of smolting returned to a simulated home stream in larger numbers as compared to salmon which had been exposed to morpholine for 40 days. The two day fish also responded to morpholine when tested with the EEG technique (Cooper, 1974).

Thus, it appears that there is a "sensitive" or "critical" period during their development for imprinting salmon. In addition, because the life cycles of each species are somewhat different the critical period for each species is not the same. Furthermore, different stocks of the same species may have different periods. For example there are stocks of coho salmon which migrate downstream after only six months in the river rather than the normal 1-1/2 years (Drucker 1972).

Steelhead trout have a similar life cycle and undergo a smolting process nearly the same as coho salmon. Experimental fish which were exposed to morpholine during a period when they exhibited downstream orientation returned to a simulated home stream as adults (see Appendix I).

Pink salmon (O. gorbuscha) and chum salmon (O. keta) fry migrate from the home stream soon after emergence, but may remain in the estuary for long periods of time. Residence in the estuary by the young fish may, thus, compensate for a short stay in the home tributary. It is unclear when imprinting would take place in these species. It may be possible to imprint the fry at the time they move downstream. However, imprinting might occur during one of the egg or alevine stages. Since the distance from the home tributary to the coast or lake is often short (involving few, if any, adjoining streams), it is also possible that imprinting occurs where the home stream meets the larger water mass (Madison et al. 1973).

Atlantic salmon smolt after 1-1/2 years although this process may be delayed if the fish have not reached a certain size. Carlin (1968) has transplanted Atlantic salmon during the smolting period and the fish returned to the new location to spawn so it would appear that this species could be artificially imprinted during smolting.

Spring and summer-run chinook salmon also migrate downstream after 1-1/2 years in the river and undergo smolting at that time. Fall-run chinook salmon fingerlings migrate out of rivers within about six months after they are hatched. These fish apparently do not smolt, but there is distinct downstream orientation. In Lake Michigan, fall-run fish are held in a hatchery for about six months from December (when they hatch) until April or May and then stocked into smolting ponds. During the spawning migration two to six years later, the fish return with good accuracy to the pond where they were released. These results indicate that chinook salmon could be imprinted just before or at the time when they are migrating downstream.

Sockeye salmon (O. nerka) normally smolt and migrate out of their nursery lake and then downstream after 1-1/2 years. However, some stocks do not smolt but instead migrate downstream as fry or fingerling after only six months in the river and others remain in freshwater for 3 or 4 years before smolting. Apparently, smolt transformation is related to size because for stocks where growth is suppressed it usually occurs later than for stocks in which growth is more rapid (Drucker 1972). This was also observed in coho salmon in the Lake Michigan experiments.

Most fish which appeared to be smolting were 100 mm or longer. Fish under this size retained their parr marks and were generally not observed to orient downstream. In Lake Michigan smolting ponds where fish were allowed to migrate downstream naturally, smolting was observed to take place over a period of about 6 to 8 weeks. The first migrants moved out of the ponds in the beginning or middle of May with others not leaving until the end of June. In contrast, in our experiments all of the fish were released when the majority appeared to be smolting. This release time should be carefully controlled because it could strongly influence the results of an imprinting study.

In summary, the imprinting process is probably rapid and occurs during the smolting period (coho salmon, spring and summer chinook salmon, sockeye salmon, Atlantic salmon, and steelhead trout) or the time the fish normally begin to migrate from the home stream (fall spawning chinook salmon, pink salmon, chum salmon). There are, of course, some exceptions to these groupings.

It is possible that imprinting is triggered by the same processes which cause smolting (increased activity of the endrocrine system). The development of this system probably coincides with growth since studies on Atlantic and sockeye salmon have shown that fish which do not reach a certain size limit will hold over in the stream another year before smolting. At the same time, size does not appear to be the only factor which controls smolting because some fish reach smolting size 6 months before smolting occurs. Since smolting occurs at about the same time every year, it is possible that photoperiod (day length) and/or temperature might, in part, also induce smolt transformation.

Thus, smolting behavior is complex, and in order to understand fully how to control the migrations of salmon (especially in regards to chemical imprinting) this behavior needs to be documented more fully for each species.

GENERAL SUMMARY

Anadromous salmon and trout stocked into ponds in tributaries of Lake Michigan return with great specificity to the stream where released. These, and other studies (Donaldson and Allen 1957; Carlin 1968; Jensen and Duncan 1971; Ricker 1972; and Vreeland et al. 1975) have implied that home stream recognition in salmon is connected with an "imprinting" process which occurs near or at the time that the young salmon start to migrate downstream. Hasler and Wisby (1951) and Hasler (1966) have theorized that during the imprinting period salmon learn the odor(s) of their home stream. They have also suggested that it might be possible to "artificially imprint" young salmon to a synthetic chemical, and then attract or decoy the salmon to a new location by scenting it with this chemical. The objective of the research described in this report was to determine if coho salmon and steelhead trout could be imprinted artificially.

The basic design for these experiments was to expose (imprint) young coho salmon and steelhead trout held at a fish hatchery to a synthetic chemical, either morpholine or phenethyl alcohol (PEA). An equal number of fish were not exposed (controls) and both groups of fish were marked and stocked into Lake Michigan after they started to smolt. During the spawning migration 18 months later, morpholine (or PEA) was metered into a stream near the location where the fish were stocked and the number of exposed and unexposed fish returning to this simulated home stream was determined. These studies were conducted from 1971-1974.

Results from these experiments have demonstrated that coho salmon which are exposed to morpholine or PEA during the smolting period will return (home) to a stream which has been scented with the appropriate chemical to spawn. Behavioral (ultrasonic tracking) and electrophysiological (EEG) studies were also conducted and have determined that imprinted fish could discriminate the chemical to which they were imprinted.

Additional experiments were conducted to determine if it was necessary to stock the fish near the simulated home stream to attract them to the stream during the spawning migration. Results from these

studies indicated that coho salmon can search an area of at least 13 km but probably not more than 48 km on either side of their stocking site to locate a simulated home stream. Ultrasonic tracking experiments have indicated that fish probably return to this area from open water by some non-chemical mechanism and apparently must return to this region before they will search for a scented stream.

Results from artificial imprinting experiments have confirmed the existence of a rapid learning chemical imprinting mechanism, long-term memory, and the use of odor cues for homing to a simulated home stream. It is evident, therefore, that it is possible to control the final stages in the migration of some species of salmonids by artificially imprinting smolts to a synthetic chemical.

Some possible applications of these findings include the following. In Lake Michigan, artificial imprinting at hatcheries reduces the need for smolting ponds, a factor which may be advantageous in terms of operating cost and survival of young fish. Artificial imprinting permits manipulation of salmon and trout runs, for example by selecting specific locations for harvest whereas fish stocked into smolting ponds return only to the river of release. Fish can also be released at locations such as metropolitan areas which do not have adequate facilities for smolting ponds. Furthermore, this technique can be utilized to improve the returns of some species such as steelhead rainbow trout which normally have to be stocked directly into the lake. Imprinting methods could also aid in many restocking programs to attract fish into suitable areas for spawning or commercial harvest. For example, on the Columbia River in Washington and Oregon, artificial imprinting could be used to identify artificial spawning channels to enhance the return to these channels.

Because artificial imprinting can be employed in the management of salmon stocks a summary of imprinting methods is provided. This summary includes detailed information about imprinting chemicals, imprinting equipment, exposure periods, stocking procedures, and calculating concentrations of imprinting chemicals.

APPENDIX I. PRELIMINARY EXPERIMENTS

In addition to the work described in this report, other experiments on coho and other salmonids have been conducted or are in progress. Preliminary information on the effects of the concentration of an imprinting chemical and length of exposure period are reported for coho salmon by Cooper (1974) and Cooper et al. (MS). In addition, experiments are being conducted to determine if salmon can be imprinted to an artificial chemical in a natural stream system and to directly compare smolting pond imprinting with artificial imprinting. Experiments have been conducted with other species including steelhead trout, brown trout, Atlantic salmon, and chinook salmon. Completed studies which have not been reported elsewhere are included in the appendix. These include preliminary experiments with coho salmon and steelhead trout.

COHO SALMON

A preliminary experiment on artificial imprinting of coho salmon was conducted in 1970-71 prior to any of the experiments described in this report (Madison et al. 1973; Cooper 1974). In the spring of 1970, 20,000 imprinted fish and 10,000 controls were released in Oak Creek. In the fall of 1971, 128 imprinted fish and 79 non-imprinted fish, a ratio of about 1:1 were recovered in Oak Creek (Table 10). This was the expected ratio if morpholine had no effect on the imprinted group.

This experiment was not directly comparable to the other experiments because procedures were different. First, census procedures were not the same. Electroshocking, which accounted for a large proportion of the fish censused in the other experiments (1972, 1973, 1974), was used only once in 1971. Second, both groups of fish were released in Oak Creek water and might have used this water for homing. Third, fish were exposed to morpholine through only one week after the onset of smolting whereas fish in the other experiments were exposed until two weeks after the onset of smolting. If the imprinting period is as critical as believed, this factor of timing could also account for the discrepancy in the results of this experiment. It is also possible that a combination of these factors could explain the results of this experiment. For example, the fish were released too early, that is before the imprinting mechanism shut off, and then became imprinted to Oak Creek water because they were released there. Madison et al. 1973 and Cooper 1974 describe and discuss this experiment in more detail.

STEELHEAD TROUT

Oak Creek Experiments

Imprinting experiments were also conducted with steelhead rainbow trout (Cooper and Scholz MS). Procedures were similar to those used for coho salmon. In May, 1972, steelhead trout fingerlings were divided into two groups with 3,000 fish/group. One group was treated with morpholine and the other group was left unexposed. Fish were exposed for 4 weeks from about mid-May to mid-June until they exhibited downstream movements. Both groups of fish were stocked at the alternate stocking location 13 km north of Oak Creek (Fig. 2).

The fish used for this experiment spawned in both the spring (March-April), and fall (September-November, at the same time as the coho); therefore, morpholine was introduced into Oak Creek in both seasons. The total number of fish recovered from fall 1972 until spring 1974 are recorded in Table 11. A total of 174 (or 5.80% of the morpholine fish stocked) were captured at Oak Creek compared to only 16 controls , a ratio of 11:1 indicating that this species will also home to a simulated home stream.

In the spring of 1973 this experiment was replicated. Two thousand exposed fish and 3,900 controls were released at the same location 13 km north of Oak Creek. Sixty-six imprinted fish and 8 nonimprinted fish, a ratio of about 15:1, were recovered at Oak Creek, confirming the results of the 1972 experiment (Table 11). Morpholine was not added to Oak Creek in the fall of 1973 (as a control for coho experiments). No steelhead from the 1972 release and only 3 imprinted and 5 nonimprinted fish from

Table 10. Results of artificial imprinting experiments conducted with coho salmon at Oak Creek, 1970.

Experimental Group	Fin Clip*	Number Released	Date Released	Number Recovered	Date Recovered	Percent of Fish Stocked
Exposed	RM	20,000	May 1970	128	Fall 1971	0.64
Controls	A	10,000	May 1970	79	Fall 1971	0.79

*See footnote Table 1 for key to abbreviations

Table 11. Results of artificial imprinting experiments with steelhead rainbow trout (Salmo gairdneri) conducted at Oak Creek in 1972 and 1973.

Experimental Group	Fin Clip*	Number Released	Date Released	Number Recovered	Date Recovered	Percent of Fish Stocked
Exposed	RP	3,000	May 72	174	1972-74	5.80
Controls	LM	3,000	May 72	16	1972-74	0.53
Exposed	D-LP	2,000	May 73	66	1973-74	3.30
Controls	D-RP	3,900	May 73	8	1973-74	0.21

*See footnote Table 1 for key to abbreviations.

the 1973 release were recovered in Oak Creek during that period.

Electrophysiological (EEG) experiments conducted with steelhead trout (Cooper and Hasler, MS) showed that morpholine-imprinted fish responded more strongly to morpholine than nonimprinted fish responded.

Manitowoc Experiments

Another experiment with steelhead trout was conducted at Manitowoc. In the summer of 1973, 10,000 morpholine-exposed and 15,000 unexposed steelhead were released at the mouth of Little Manitowoc River. During the fall of 1974, concurrently with the coho experiments, morpholine was metered into Little Manitowoc River. These fish were fall spawners and so the river was not scented in the spring. As in the other experiments conducted at Manitowoc, Little Manitowoc River and 27 other locations were monitored in order to determine the accuracy with which imprinted fish return to a simulated home stream.

In the fall of 1974, 138 imprinted fish were captured in Little Manitowoc River compared to only 11 controls, a ratio of 19.5:1 (Table 12). In contrast, 20 imprinted fish and 92 controls were recovered at other locations. These results indicate a high degree of accuracy in the homing of imprinted fish and document a considerable amount of straying in the control group. Additional returns are expected in the fall of 1975 and 1976.

Table 12. Recoveries of imprinted and nonimprinted steelhead rainbow trout in 1974.

Recovery Location	Number Recovered		Recovery Location	Number Recovered	
	Imprinted	Control		Imprinted	Control
1. Schylers	-	-	15. Manitowoc Rapids	1	7
2. Bear Creek	-	1	16. Hika	-	-
3. Stony Creek	-	1	17. Pigeon River	-	-
4. Ahnapee River	4	14	18. Sheboygan - North Pt.	-	-
5. Three Mile Creek	-	2	19. Sheboygan River	-	1
6. Kewaunee River	-	1	20. Sheboygan Power Plant	-	1
7. Kewaunee Power Plant	2	13	21. Port Washington	4	3
8. Point Beach Power Plant	2	8	22. Milwaukee Harbor	-	-
9. Molash Creek	-	-	23. Milwaukee Coast Guard	-	-
10. Two Rivers Breakwater	6	27	24. Lakeside Power Plant	-	-
11. East Twin River	-	1	25. Oak Creek	-	-
12. West Twin River	-	1	26. Root River	-	-
13. Manitowoc Bay	-	-	27. Pikes Creek	1	-
14. Little Manitowoc River	138	11	28. Kenosha Breakwater	-	-

APPENDIX II. SAMPLE CALCULATIONS

CALCULATION 1: Raceway or Stream Flow (Amount of Flow over a Broad-Crested Weir).

FORMULA: $TF = W\sqrt{C \cdot D^3}$

Where: TF is the total flow (ml/sec)
W is the width of the dam (cm)
C is a constant, the acceleration due to gravity (980.7 cm/sec^2)
D is the depth of the water flowing over the weir (cm)

Step 1: Measure width of wier and average depth of water flowing over the weir. For purposes of this sample calculation, assume that:

width = 95 cm
average depth (average of measurements taken at 10 cm intervals) = 4.0 cm

Step 2: Substitute known values in the formula and solve for TF.

TF = unknown
W = 95.0 cm
D = 4.0 cm
C = 980.7 cm/sec^2

The formula now reads:

$TF = 95\ cm\sqrt{(980.7\ cm/sec^2)\ (4.0\ cm)^3}$

Step 3: Complete the arithmetic calculations to get a value for TF.

a. cube the depth: $(4.0\ cm)^3 = 64\ cm^3$
b. multiply 980.7 cm/sec^2 x 64 cm^3 = 62,764.8 cm^4/sec^2
c. take the square root of 62,764.8 cm^4/sec^2 = 250.53 cm^2/sec
d. multiply 250.53 cm^2/sec x 95 cm = 23,800 cm^3/sec

Thus; TF = 23,800 cm^3/sec

Step 4: Convert the flow rate obtained in Step 3 to l/sec in order to use it for calculating chemical concentrations.

a. 1 cm^3/sec = 1 ml/sec
Therefore, 23,800 cm^3/sec = 23,800 ml/sec
b. 1,000 ml = 1 liter
Therefore, 23,800 ml/sec = 23.8 l/sec

CALCULATION 2: Drip Concentration (Amount of Chemical to be Added to the Reservoir Bottle)

FORMULA: $DC = \frac{SSC \times FR}{DR}$

Where: DC = Drip Concentration (g/l)
SSC = Steady State Concentration (g/l)
FR = Flow Rate (l/sec)
DR = Drip Rate (l/sec)

Step 1: Assemble known values.

a. Steady State concentration is a predetermined value. For morpholine it is 5×10^{-5} mg/l.
b. Flow rate has already been calculated from the previous formula. In our example it is 23.8 l/sec.

Step 2: Determine the drip rate from calibration of the imprinting pump. To calibrate the pump, the end of the tubing is placed in a graduated cylinder and the amount of solution after one hour is measured. A sample calibration is given below.

Trial Number	Drip Rate (ml/hr) with Pump Speed Control Set At 0.5	1.0	1.5	2.0	2.5
1	8	21	50	58	85
2	12	18	40	58	76
3	10	16	48	58	66
Average	10	18	46	58	77

For purposes of this sample calculation, assume that the drip rate is set at 10 ml/hr (pump speed 0.5).

Step 3: Make Unit conversions.

a. SSC is given in mg/l and needs to be converted to g/l in order to be used in the equation.

1000 mg = 1 g

Therefore, 5×10^{-5} mg/l $= 5 \times 10^{-8}$ g/l

b. DR is calibrated in ml/hr and needs to be converted to l/sec.

1,000 ml = 1 liter
3,600 sec = 1 hour

Therefore, $\frac{10 \text{ ml/hr}}{1000 \text{ ml/l}} = .01$ l/hr

$\frac{.01 \text{ l/hr}}{3600 \text{ sec/hr}} = .00000278$ l/sec $= 2.78 \times 10^{-6}$ l/sec

Step 4: Substitute known values in formula.

$$DC = \frac{(5 \times 10^{-8} \text{ g/l}) \times 23.8 \text{ l/sec}}{2.78 \times 10^{-6} \text{ l/sec}}$$

Step 5: Complete the arithmetic calculations.

$$DC = \frac{(5 \times 10^{-8}) \times 23.8}{2.78 \times 10^{-6}}$$

$$= \frac{119.0 \times 10^{-8}}{2.78 \times 10^{-6}}$$

$$= 42.8 \times 10^{-2} \text{ g/l} = 0.428 \text{ g/l}$$

Step 6: The drip concentration is the amount of chemical that is added to 1 liter of water. Since a 19 l bottle was used as a reservoir for the chemical, the drip concnetration had to be multiplied by the volume of the bottle.

Therefore: 0.428 g/l x 19.0 l = 8.13g

Step 7: If the chemical comes in a liquid form, it is necessary to convert grams to milliliters. This conversion depends on the density of the liquid Since the density of morpholine is about equal to 1, one g of morpholine is equal to 1 ml of morpholine.

Therefore: 8.13g of morpholine = 8.13 ml of morpholine

This is the amount of morpholine which is added to a 19 liter reservoir bottle in order to achieve a steady state concentration of 1×10^{-5} mg/l.

CALCULATION 3: Conversions necessary for calculating steady state concentrations in English units.

FORMULA: a. $\text{Flow Rate (in cfs)} = \frac{\text{Flow Rate in l/sec}}{28.32}$

b. $\text{Drip Rate (in U.S. gal/hr)} = \frac{\text{Drip Rate in ml/hr}}{3,784}$

LITERATURE CITED

Allen, R. and T. Meiken
1973. An evaluation of the Priest Rapids chinook salmon spawning channel, 1963-1971. Washington Dept. Fish. Tech. Rep. No. 11.

Anderson, G. A.
1962. Three portable feeders for metering chemicals into streams for control of the sea lamprey. U.S. Fish Wild. Serv., Prog. Fish-Cult. 24:190-192.

Applegate, V.C., J. H. Howell, J.W. Moffett, B.G. Tomson and M.A. Smith
1961. Use of 3-trifluoromethyl-4 nitrophenol as a selective sea lamprey larvicide. Great lakes Fish. Comm. Tech. Rep. No. 1. 35p.

Avery, E.L.
1974. Reproduction and recruitment of anadromous salmonids in Wisconsin tributaries of Lake Michigan. Wisc. Dept. Nat Resour. 32p.

Brett, J.R. and C. Groot
1963. Some aspects of olfactory and visual responses in Pacific salmon. J. Fish. Res. Bd. Canada 20:287-303.

Brett, J.R. and D. MacKinnon
1954. Some aspects of olfactory perception in migrating adult coho and spring salmon. J. Fish Res. Bd. Canada 11:310-318.

Carlin, B.
1968. Salmon conservation, tagging experiments, and migrations of salmon in Sweden. Lecture series, The Atlantic Salmon Assoc., Montreal.

Cooper, J.C.
1974. Olfactory imprinting and memory in salmonids. Ph.D. Thesis, Univ. of Wisconsin, Madison.

Cooper, J.C. and A.D. Hasler
1973. II. An electrophysiological approach to salmon homing. Fish Res. Bd. Canada Tech. Rep. No. 415.

1974. Electrophysiological evidence for retention of olfactory cues in homing coho salmon. Science 183:336-338.

1975. Morpholine as an olfactory stimulant. Science 187:82.

In Press. Physiological studies on morpholine for imprinting coho salmon. J. Fish. Res. Bd. Canada.

Cooper, J.C., G.F. Lee and A.E. Dizon
1974. An evaluation of the use of the EEG technique to determine chemical constituents in homestream waters. Trans. Wis. Acad. Sci., Arts, Lett. 62:165-172.

Cooper, J.C. and A.T. Scholz
In Press. Homing of artificially imprinted steelhead (rainbow) trout, Salmo gairdneri. J. Fish. Res. Bd. Canada.

Cooper, J.C., A.T. Scholz, R.M. Horrall and A.D. Hasler
In Press. Experimental confirmation of the olfactory hypothesis with artifically imprinted coho salmon (Oncorhynchus Kisutch). J. Fish. Res. Bd. Canada.

Corbett, D.M. and others
1953. Stream gaging procedure: a manual describing methods and practices of the Geological Survey. U.S. Geol. Surv. Water Supply Paper 888. 245p.

Dizon, A.E., R.M. Horrall and A.D. Hasler
1973. Long-term olfactory "memory" in coho salmon, Oncorhynchus kisutch. Fish. Bull. 71(1):315-317.

Donaldson, R. and G.H. Allen
1957. Return of silver salmon Oncorhynchus Kisutch (Walbaum) to point of release. Trans. Amer. Fish. Soc. 87:13-22.

Drucker, B.
1972. Some life history characteristics of coho salmon of the Karluk River System, Kodiak Island, Alaska. Fish Bull. 70:79-99.

Ebel, W.J., D.L. Park and R.C. Johnsen
1973. Effects of transportation on survival and homing of Snake River chinook salmon and steelhead trout. Fish. Bull. 71:549-563.

Fessier, J.
1974. Determination of factors limiting efficiency of hatchery production and the population characteristics and life history of Deschutes River summer steelhead. Oregon Wild. Comm. Fed. Aid Prog. Rep. in Fish. 1974, F-88-R. 27p.

Fulton, L.A.
1968. Spawning areas and abundance of chinook salmon (O. tshawytscha) in the Columbia River Basin -- past and present. U.S. Fish and Wild. Serv. Spec. Sci. Rep. No. 571. 26p.

Hara, T.J.
1970. An electrophysiological basis for olfactory discrimination in homing salmon: a review. J. Fish. Res. Bd. Canada 27:565-586.

Harden-Jones, F.R.
1968. Fish Migration. St. Martin's Press, New York. 325p.

Hasler, A.D.
1966. Underwater Guideposts - Homing of Salmon. Univ. of Wisconsin Press, Madison. 155p.

Hasler, A.D. and W.J. Wisby
1951. Discrimination of stream odors by fishes and relation to parent stream behavior. Amer. Natur. 85:223-238.

1959. Repelling fish by treatment with potassium phenylacetate. U.S. Patent No. 2,880,113. March 31, 1959.

Hess, E.H.
1973. Imprinting. Early experience and the developmental psychobiology of attachment. Van Nostrand Reinhold, New York. 472p.

Hoar, W.S.
1951. The behavior of chum, pink and coho salmon in relation to their seaward migration. J. Fish. Res. Bd. Canada 8:241-263.

1958. The evolution of migratory behavior among juvenile salmon of the genus Oncorhynchus. J. Fish. Res. Bd. Canada 15:391-428.

Idler, D.R., J.R. McBride, R.E. Jones and N. Tomlinson
1961. Oflactory perception in migrating salmon. II. Studies on a laboratory bioassay for homestream water and mammalian repellant. Can. J. Biochem. Physiol. 39:1575-1584.

Jensen, A. and R. Duncan
1971. Homing of transplanted coho salmon. The Prog. Fish-Cult. 33:216-218.

Kleerekoper, H.
1969. Olfaction in fishes. Indiana Univ. Press, Bloomington. 222p.

Kubitschek, H.E.
1954. Modifications of the chemostat. J. Bacteriol. 67:254-255.

Madison, D.M., R.M. Horrall, A.B. Stasko and A.D. Hasler
1972. Migratory movements of adult sockeye salmon (Oncorhynchus nerka) in coastal British Columbia as revealed by ultrasonic tracking. J. Fish. Res. Bd. Canada 29:1025-1033.

Madison, D.M., A.T. Scholz, J.C. Cooper and A.D. Hasler
1973. I. Olfactory hypotheses and salmon migration: a synopsis of recent findings. Fish. Res. Bd. Canada Tech. Rep. No. 414.

Mayr, E.
1974. Behavior programs and evolutionary strategies. Amer. Sci. 62:650-659.

Mills, D.H. and P.E. Shackley
1971. Salmon smolt transportation experiments on the Canon River System, Ross-Shire. Freshwater and Salmon Fisheries Research. 40:8p.

Peck, J.W.
1970. Straying and reproduction of coho salmon, Oncorhynchus kisutch, planted in a Lake Superior tributary. Trans. Amer. Fish. Soc. 99:591-595.

Rayner, W.H. and M.O. Schmidt
1957. Surveying. D. Van Nostrand Company, Inc. 916p.

Rich, W.H. and H.B. Holms
1929. Experiments in Marking young chinook salmon of the Columbia River. Fish. Bull. 44:215-264.

Ricker, W.E.
1972. Hereditary and environmental factors affecting certain salmonid populations. 27-160p. In Simon, R.C. and P.A. Larkin (eds). The Stock Concept in Pacific Salmon. H.R. MacMillan Lectures in Fisheries. Univ. of British Columbia. 231p.

Scheer, B.T.
1939. Homing instinct in salmon. Quart. Rev. Biol. 14(4):408-420.

Scholz, A.T., J.C. Cooper, D.M. Madison, R.M. Horrall, A.D. Hasler, A.E. Dizon and R. Poff
1974. Olfactory imprinting in coho salmon: behavioral and electrophysiological evidence. Proc. 16th Conf. Great Lakes Research, Huron, Ohio. 143-153p.

Smith, B.R., J.J. Tibbles and B.G. Johnson
1974. Control of the sea lamprey (Petromyzon marinus) in Lake Superior, 1953-70. Great Lakes Fish. Comm. Tech. Rep. 26. 60p.

Steffel, S.
1972. Olfactory responses of 3-year-old coho salmon (Oncorhynchus kisutch). Unpublished. Reported in Cooper and Hasler 1973.

Stuart, T.A.
1958. Marking and regeneration of fish. Dep. Agr. and Fish, Scotland, Freshwater and Salmon Fish. Res. 22:1-14.

Tarrant, R.M.
1966. Thresholds of perception of eugenol in juvenile salmon. Trans. Amer. Fish. Soc. 95:112-115.

Teichmann, H.
1959. Uber die Leistung des Geruchssinnes bein Aal (Anguilla anguilla). Vorgl. Physiol. 42:206-254.

1962. Die Chemorezeption des Fische. Ergebn. Biol. 25:177-205.

Terhune, L.D.
1968. Free-floating current followers. Fish. Res. Bd. Canada Tech. Rep. 85. 21p.

Urquhart, L.C.
1957. Civil Engineering Handbook. McGraw-Hill Book Company, Inc. 1002p.

U.S. Geological Survey
Annual. Water resources data reports for individual states.

Vannard, J.K.
1961. Elementary fluid mechanics. Wiley. New York. 437p.

Vreeland, R.R., R.J. Wahle and A.H. Arp
1975. Homing behavior and contribution to Columbia River fisheries of marked coho salmon released at two locations. Fish. Bull. 73(4): 717-725.

Walker, J.C.
1967. Odor discrimination in relation to homing in Atlantic salmon. M.S. Thesis, Univ. of New Brunswick. 126 p.

Wisby, W.J.
1952. Oflactory responses to fishes as related to parent stream behavior. Ph.D. Thesis, Univ. of Wisconsin, Madison. 41 p.

Wisby, W.J. and A.D. Hasler
1954. Effect of olfactory occulsion om migrating silver salmon (O. Kisutch). J. Fish. Res. Bd. Canada 11:472-478.

Wisconsin Department of Natural Resources
Unpublished. Internal Reports.